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FURTHER INVESTIGATIONS ON THE HYMENOPTEROUS PARASITES OF *CEROPLASTES RUBENS* IN JAPAN*

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INTRODUCTION

As far as can be ascertained *Ceroplastes rubens* Maskell reached Japan without any of the natural enemies attacking it in its native country. Furthermore this scale insect attacks no less than 150 species of plants in Japan. Thus *Ceroplastes rubens* has become the first most important pest in horticulture in Japan, since not a single effective chemical control method applicable to this pest has ever been established. The wide range of host plants indicates the difficulty of its control even if the scales in the commercial orchards are satisfactorily controlled by some chemical methods. Therefore, there is also urgent need for the complete destruction of *Ceroplastes rubens* which are attached to a number of plant species in various places near or surrounding the commercial orchards.

Since 1923, as written in the previous report (1949), not a single investigation has ever been made on the native parasites of this injurious scale insect up to the year 1946, when I noticed for the first time a progressive decrease in the population of *Ceroplastes rubens* in N. Kyushu and this phenomenon was ascribed to the activity of a parasitic Hymenoptera.

Increasing requirements in recent years for the control of this scale especially in Honshu and Shikoku emphasize the necessity of developing new approaches to the problem. Among the suggestions advanced is that the possibility of biological control by means of this native parasite should receive adequate attention.

* The expenses of the present study were born in part by a grant from the Ministry of Education.

What was known of the parasites of *Ceroplastes rubens* is Japan previous to the year 1947 has been reported in the previous paper (1949). This paper includes an account of my research for the native key parasite performed in 1949, 1950 and an early part of 1951.

Re-examination of the Key Parasite

In Japan two species of the genus *Anicetus* have been recognized by Dr. T. Ishii (1928): *Anicetus annulatus* Timberlake and *A. ceroplastis* Ishii. When the key parasite of *Ceroplastes rubens* was first discovered in Kyushu, I determined it as *Anicetus annulatus* Timberlake with some hesitation, because at that time not a single available specimen of *A. annulatus* from U. S. A. was before me. During recent years doubts have been growing as to whether the key parasite is *A. annulatus*. Therefore, I sent my material to Dr. H. Compere in 1950 and begged him to compare them with the type of *A. annulatus*. Dr. Compere was so kind as to give me some information about the species and spared me some specimens of *A. annulatus* determined by him for my comparative study. On the other hand, Mr. Y. Miyamoto compared my specimens with the type of *A. ceroplastis* Ishii and informed me that the key parasite was identical with the type of *A. ceroplastis*, the only difference between the two species was seen in the length of the ovipositor and the original description made by Ishii was incorrect with respect to the ovipositor length. I was deeply astonished at this fact. The reason was that the original description made by Dr. Ishii did not agree with the character of the key parasite. Unfortunately the original description of *A. ceroplastis* is so inaccurate that it is almost impossible to detect the specimens of *A. ceroplastis* among the material of the genus *Anicetus* with the aid of his description.

Because of the necessity for accurate knowledge of this key parasite and because of the widespread interest in the race problem, I undertook the re-examination of the key parasite and came to the conclusion that there may be found many significant differences separating the two species besides the length of the ovipositor (Tables 1 and 2).

Table 1. Comparison of *A. ceroplastis* and *A. annulatus* (female sex).

annulatus	*ceroplastis*
Head, seen from above, about 1.7-times as broad as long.	Head, seen from above, about twice as broad as long.
Fronto-vertex 2.5 times as long as wide at the ocelli.	Fronto-vertex about three-times as long as broad.
Antennal scape subtriangular, maximum length: maximum width=1.5:1 (Fig. 1, B).	Antennal scape somewhat trapezoidal, maximum length: maximum width=1.4:1 (Fig. 1, D).
Mesoscutum slightly longer than scutellum.	Mesoscutum slightly shorter than or as long as scutellum.
Thorax with the notum moderately convex.	Thorax with the notum much more convex.
Thorax—length : width : hight = 20 : 14 : 11.	Thorax—length : width : hight = 20 : 18 : 17.
Pronotum without marking.	Pronotum with a brownish round spot.
Scutellum wider than long (Fig. 1, A).	Scutellum as long as wide (Fig. 1, c).
Scutellum with two pairs of still stouter longer black bristles, one pair near the apex, the other but slightly further apart just behind the middle, the disk also with a few fine hairs (Fig. 1, A).	Scutellum with many bristles and hairs, two pairs of stouter bristles near the apex (Fig. 1, C).

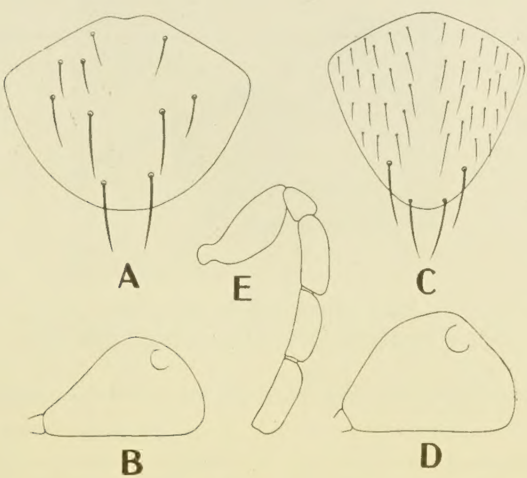

Fig. 1. *Anicetus annulatus* (A, B) and *A. ceroplastis* (C, D, E).
A, C: Scutellum (♀). B, D: Antennal scape (♀).
E: Five basal antennal segments (♂).

Metapleura with an oval to oblong spot on upper part.	Metapleura without an oval to oblong spot.
Hairs on mesoscutum pale coloured.	Hairs on mesoscutum all black.
Hairs on fore wing different (Fig. 2, B).	Hairs on fore wing also different (Fig. 2, A).
Hind wing narrow—length/width = 4.75.	Hind wing broader—length/width = 3.33.
Ovipositor barely produced.	Ovipositor producing one-fifth the length of abdomen.
Abdomen slightly wider than thorax.	Abdomen almost as wide as thorax.
Hind tibia with two annuli, the first situated close to the base, the second just beyond the middle.	Hind tibia with three annuli, the first situated at the base.
General colour ochraceous orange.	General colour much darker, more strongly violaceously reflecting.

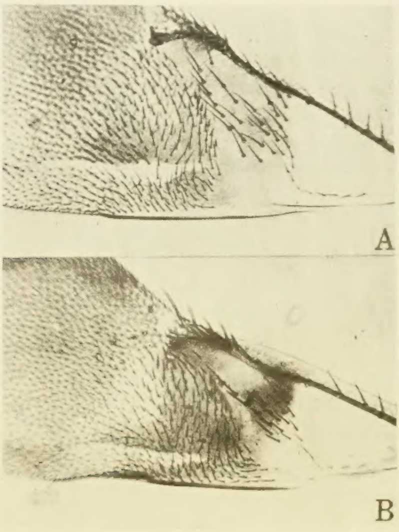

Fig. 2. Basal half of fore wings.
A: *Anicetus ceroplastis* (♀). B: *A. annulatus* (♀).

Table 2. Comparison of *A. ceroplastis* and *A. annulatus* (male sex).

annulatus	*ceroplastis* (new to science)
Cheeks considerably longer than eyes.	Cheeks slightly shorter than eyes.
Antenna—the first funicle segment somewhat longer than the second to fourth. The first a half longer than wide. The fifth about twice as long	First funicle segment very slightly longer than the second, about as long as third to fourth. The first about three-times as long as wide.

as wide. The second to fifth each of deeply incised at the articulations so that the second to fourth are subtriangular. Club nearly as long as the last two funicle segments taken together.	The fifth more than three-times as long as wide. The second to fourth parallel-sided, but triangular in shape. Club shorter than the last two funicle segments taken together (Fig. 1, E).
Mesoscutum much longer or hardly a fourth wider than long.	Mesoscutum twice as broad as long.
Scutellum much shorter than mesoscutum.	Scutellum distinctly longer than mesoscutum.
Abdomen hardly wider than thorax.	Abdomen slightly wider than thorax.
Fore and mid-formora pale yellow. Basal third of the mid-tibiae fuscous.	Basal half of fore femora fuscous. Mid-femora fuscous except the base and the apex. Basal portion of mid-tibiae not fuscous.
Hind femora fuscous. Apex of all tarsi black.	Hind femora blackish except the apical portion. Apical tarsal segment of fore and hind legs brownish. Apex of mid-tarsi brownish.
Hosts: *Coccus hesperidum, Coccus pseudomagnoliarum, Eucalymnatus tesselatus, Pulvinaria* sp., *Saissetia hemisphaerica.*	Hosts: *Ceroplastes rubens, Ceroplastes ceriferus.*

Allotype (♂) and 52 paratopotypes (♂♂) of *Anicetus ceroplastis*—16. vi. 1950, Fukuoka, Prov. Chikuzen, Kyushu, reared by K. Yasumatsu from *Ceroplastes rubens.*

PERCENTAGE OF PARASITISM OF *Ceroplastes rubens* BY *Anicetus ceroplastis* IN VARIOUS DISTRICTS OF JAPAN

It was planned to continue the survey for three years (1949, 1950, 1951) to determine what species of parasites were present and most important in various districts of Japan. It was believed that such a survey would also yield information that would enable us to better utilize the native parasites known to attack *Ceroplastes rubens.* The results are summarized in Table 3.

Table 3. Parasitism of *Ceroplastes rubens* by *Anicetus ceroplastis* in different localities of Japan observed in 1949, 1950 and 1951.

Host plants of *Ceroplastes rubens*	Localities	No. of *C. rubens*	No. of *A. ceroplastis* emerged	Percentage of parasitism
Ilex integra	Mitaka-gun, Fuk.	2174	0	0.00
Diospyros Kaki	Mitaka-gun, Fuk.	1765	0	0.00
Citrus Unshu	Mitaka-gun, Fuk.	132	0	0.00
Citrus Unshu	Oi-gun, Fuk.	30	0	0.00
Diospyros Kaki	Onifu-gun, Fuk.	5976	0	0.00
Citrus Unshu	Onifu-gun, Fuk.	326	0	0.00

Ilex integra	Onifu-gun, Fuk.	579	0	0.00
Citrus Unshu	Onifu-gun, Fuk.	731	0	0.00
Diospyros Kaki	Onifu-gun, Fuk.	992	0	0.00
Citrus Unshu	Uchiura, Si.	313	0	0.00
Eurya japonica var. *montana*	Kanaya, Si.	272	0	0.00
Ilex integra	Kanaya, Si.	256	0	0.00
Thea sinensis	Kanaya, Si.	154	0	0.00
Diospyros Kaki	Kanaya, Si.	443	0	0.00
Gardenia jasminoides var. *grandiflora*	Kanaya, Si.	227	0	0.00
Citrus Unshu	Kanaya, Si.	849	0	0.00
Citrus Unshu	Chita-gun, Ai.	451	0	0.00
Citrus Unshu	Chita-gun, Ai.	260	0	0.00
Citrus Unshu	Chita-gun, Ai.	1500	0	0.00
Citrus Unshu	Atsumi-gun, Ai.	160	0	0.00
Citrus Unshu	Atsumi-gun, Ai.	416	0	0.00
Eurya japonica var. *montana*	Chita-gun, Ai.	950	0	0.00
Eurya japonica var. *montana*	Chita-gun, Ai.	315	0	0.00
Ilex integra	Chita-gun, Ai.	672	0	0.00
Ilex integra	Chita-gun, Ai.	450	0	0.00
Citrus Unshu	Unebi-machi, Na.	5080	0	0.00
Diospyros Kaki	Ryumon, Wa.	89692	0	0.00
Citrus Unshu	Ryumon, Wa.	153310	0	0.00
Daphniphyllum macropodum	Takatsuki, Os.	9	0	0.00
Camellia japonica var. *hortensis*	Minami-kawachi, Os.	34	0	0.00
Diospyros Kaki	Takatsuki, Os.	329	0	0.00
Diospyros Kaki	Minami-kawachi, Os.	52	0	0.00
Varia	Ikeda, Os.	2230	0	0.00
Gardenia jasminoides var. *grandiflora*	Ikeda, Os.	211	0	0.00
Ilex latifolia	Ikeda, Os.	67	0	0.00
Citrus Unshu	Ikeda, Os.	70	0	0.00
Eurya sp.	Ikeda, Os.	23	0	0.00
Cercidiphyllum japonicum	Ikeda, Os.	92	0	0.00
Euonymus alatus	Ikeda, Os.	410	0	0.00
Fastia japonica	Ikeda, Os.	104	0	0.00
Daphniphyllum macropodum	Ikeda, Os.	258	0	0.00
Diospyros Kaki	Ikeda, Os.	398	0	0.00
Camellia japonica var. *hortensis*	Ikeda, Os.	268	0	0.00
Ilex integra	Ikeda, Os.	2024	0	0.00
Ilex integra	Nishinomiya, Hy.	288	0	0.00
Pyracantha angustifolia	Nishinomiya, Hy.	293	0	0.00

Citrus Unshu	Akomachi, Hy.	3925	0	0.00
Citrus Unshu	Sumoto, Hy.	559	0	0.00
Diospyros Kaki	Okayama, Ok.	9607	0	0.00
Thea sinensis	Mitsuki-gun, Hi.	969	0	0.00
Citrus Unshu	Mitsuki-gun, Hi.	324	0	0.00
Diospyros Kaki	Mitsuki-gun, Hi.	5525	0	0.00
Diospyros Kaki	Hiroshima, Hi.	27825	0	0.00
Citrus Unshu	Jigoze, Hi.	490	0	0.00
Diospyros Kaki	Mino-gun, Sim.	46	0	0.00
Diospyros Kaki	Anno-gun, Sim.	18	0	0.00
Citrus sp.	Anno-gun, Sim.	111	0	0.00
Camellia Sasanqua	Izumo, Sim.	415	0	0.00
Varia	Hikawa-gun, Sim.	684	0	0.00
Ilex integra	Hikawa-gun, Sim.	472	0	0.00
Diospyros Kaki	Hikawa-gun, Sim.	167	0	0.00
Camellia japonica var. *hortensis*	Hikawa-gun, Sim.	24	0	0.00
Citrus Unshu	Hikawa-gun, Sim.	255	0	0.00
Ilex serrata var. *Sieboldi*	Hikawa-gun, Sim.	575	0	0.00
Citrus Unshu	Yanai, Ya.	45	0	0.00
Camellia japonica var. *hortensis*	Yanai, Ya.	100	0	0.00
Diospyros Kaki	Naka-gun, Ya.	200	0	0.00
Citrus Unshu	Iwakuni, Ya.	63	0	0.00
Diospyros Kaki	Hagi, Ya.	28	0	0.00
Citrus Unshu	Hagi, Ya.	1183	0	0.00
Citrus Unshu	Oshima, Ya.	7726	0	0.00
Citrus Unshu	Matsuyama, Eh.	456	7	1.53
Fortunella sp.	Sasaguri, Fu.	3352	1740	over 52.33
Citrus Unshu	Tachibana, Fu.	567	140	over 24.67
Varia	Kokura, Fu.	1133	316	over 27.90
Citrus Unshu, varia	Tanushimaru, Fu.	573	260	over 45.37
Citrus Unshu	Fukuma, Fu.	539	220	over 40.81
Ilex Oldhami	Orio, Fu.	739	182	over 29.23
Fortunella sp.	Miyajidake, Fu.	783	118	over 15.07
Citrus Unshu	Takeo, Sa.	1126	204	over 18.11
Citrus Unshu	Saga, Sa.	546	152	over 27.83

Fu.: Fukuoka Prefecture, Kyushu. Sa.: Saga Pref., Kyushu. Fuk.: Fukui Pref., Honshu. Si: Shizuoka Pref., Honshu. Ai.: Aichi Pref., Honshu. Wa.: Wakayama Pref., Honshu. Os.: Osaka Pref., Honshu. Hy.: Hyogo Pref., Honshu. Ok.: Okayama Pref., Honshu. Hi.: Hiroshima Pref., Honshu. Sim.: Shimane Pref., Honshu. Ya.: Yamaguchi Pref., Honshu. Eh.: Ehime Pref., Shikoku.

Besides the materials summarized in the table I have had the good fortune of examining other material from Isahaya, Nagasaki

Prefecture, Nozumura, Yatsushiro-gun, Kumamoto Prefecture, Ichiki-machi, Kajiki-machi and Tarumi-machi, Kagoshima Prefecture and numerous places of Fukuoka Prefecture. In the fall of 1949 I visited Nozumura where six citrus orchards were situated side by side, each differing in tree and cultural conditions together with the topographical position. Several years before these citrus orchards were said to have been heavily infested with *Ceroplastes rubens*, but during the past three years there has been a remarkable decrease in the population of the scales. My careful examination revealed that out of six orchards five were quite free from *Ceroplastes rubens* and in the remaining one orchard a number of exit holes of the parasites could be recognized, though not heavily infested with the scales.

The survey covering these years showed that only a parasite, *Anicetus ceroplastis*, was exclusively the most numerous parasite of *Ceroplastes rubens* in Kyushu, accounting for over 95 per cent of all parasites reared from the host scales. And it is very interesting to note that this parasite was not found in any district of Honshu and Shikoku except in some places where it seemed to have been imported from Kyushu several years ago together with the host scales attached to young citrus plants. These findings led to colonization experiments with *Anicetus ceroplastis* in several localities in Honshu, Shikoku and Kyushu where this parasite is not found or the percentage of parasitism is very low.

On a Possible New Race of *Anicetus ceroplastis* Parasitic on *Ceroplastes rubens*

In 1941 Professor H. S. Smith expressed the following valuable opinion concerning the biological control of insect pests: "Before the possibilities of the biological control method of pest suppression have been exhausted, the probable existence of races having varying characteristics in parts of a parasite's range will have to be given consideration in connection with the attempt to establish a species in a new habitat." Several especially interesting examples of racial segregation among Hymenopterous insects are given as follows: a Korean race of *Tiphia popilliavora* Rohwer parasitic on *Popillia japonica* Newman, a New Jersy race of *Macrocentrus ancylivorus* Rohwer parasitic on *Grapholitha molesta*

Busck, a Chinese race of *Aspidiotiphagus citrinus* (Crawford) parasitic on *Aonidiella aurantii* (Maskell), a Formosan race of *Prospaltella perniciosi* Tower and a Chinese race of *Comperiella bifasciata* Howard parasitic on the same scale. Thus in 1950 Dr. S. E. Flanders wrote "the discovery that *Comperiella bifasciata*, *Prospaltella perniciosi*, and *Aspidiotiphagus citrinus* consist of host-limited races finally establishes the principle that in parasite importation programs cognizance must be taken of this phenomenon. The host specificity of a parasite species in one region is not necessary the same as that in another."

The remarkable fact that during the past few years there has been a progressive decrease in the population of *Ceroplastes rubens* in Kyushu and the existence of the key parasite, *Anicetus ceroplastis*, has been of utmost importance in destroying this scale which might be of extreme importance in the biological control problem of this scale in various places in Honshu and Shikoku.

Anicetus ceroplastis was hitherto known as a parasite of *Ceroplastes ceriferus* (Anderson), occurring both in Honshu and Kyushu. The field evidence proved that *Ceroplastes rubens* in Honshu and Shikoku were entirely free from the parasitization of *Anicetus ceroplastis*. Further the evidence that is seen extensively in Kyushu is quite striking, and this phenomenon has appeared in *Anicetus ceroplastis* very shortly before 1946. This may have been due to the possibility that the gene for parasitizing *Ceroplastes rubens* arose by mutation. These circumstances support the fact that more than one mutant must have arisen at practically the same time at different foci throughout Kyushu except for some localities, because it is scarcely conceivable that spread of this mutant or a new race of *Anicetus ceroplastis* would have occurred from a single mutant or focus (Fukuoka Prefecture) and spread to Saga, Kumamoto, Nagasaki, Kagoshima, Oita and probably to Miyazaki Prefectures.

Fluctuation in the populations of *Ceroplastes rubens* are often influenced by such factors as climatic, tree and some cultural conditions. During the war almost all the orchards in Japan were neglected, and the fruit trees received little or no proper attention for a considerable period. There has been no noticeable difference in climate on Honshu, Shikoku and Kyushu where the scale has had no insecticidal control during these years. Notwith-

standing only in Kyushu the population of *Ceroplastes rubens* has reduced prominently in recent years.

Taking these in consideration, it should be noted at this point that there may be no objection to the claim that a progressive decrease in the population of this scale in Kyushu has been shown in almost all cases by the activity of a new race of *Anicetus ceroplastis*.

Emergence and Number of Generations of *Anicetus ceroplastis*

A summary of the data upon the emergence and number of generations of *Anicetus ceroplastis* for 1949 and 1950 in Fukuoka is given in Figure 3. The hibernation period is spent as a larva within the scale insect. The full-grown larvae may be found even at the later part of March. In April and the first part of May some larvae are already pupated. Adult *Anicetus ceroplastis* starts emerging the later part of May and continues until the first part of July. For the second generation, emergence starts the middle part of August and continues until the middle part of October.

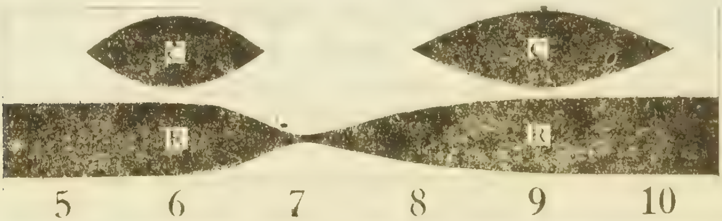

Fig. 3. Relation between *Anicetus ceroplastis* and its host, *Ceroplastes rubens*, in Kyushu. Somewhat schematic. C: Emergence of *Anicetus ceroplastis*. The extremes of each area represent approximately the first and last periods of emergence, while the widest portion represents the peak of emergence. R: Existence of *Ceroplastes rubens* scales which will become hosts of the parasite, *Anicetus ceroplastes*, in warmer seasons. 5: May. 6: June. 7: July. 8: August. 9: September. 10: October.

In Japan *Ceroplastes rubens* has but one generation a year. The oviposition occurs in May, June, July, or even in August. In Kyushu the hatching of the young individuals takes place

continuously from about the middle of May to August, a period of about three months or more. Next, it is interesting to speculate on why *Anicetus ceroplastis* persists year after year in Kyushu. Experiments have shown that the parasite will oviposit in scales almost any stages of development except the youngest one which is still uncovered by wax. Thus adult parasites of the first generation must search for the scales that are adequate enough for oviposition, but those emerged comparatively later must have a period of waiting before oviposition until the new scale larvae become available. The longevity of *Anicetus ceroplastis* seems to be sufficient to enable them to bridge the gap between their emergence and the occurrence of scales of the new generation that are large enough for oviposition. With the appearance of the adequate host larvae (perhaps the middle or later part of July and the first part of August) adult *Anicetus ceroplastis* begins to parasitize. The irregularity of oviposition or growth in the part of *Ceroplastes rubens* appears to answer one of the main reasons for the success of *Anicetus ceroplastis* to maintain itself in early summer season. Adult parasites of the second generation can find the host larvae without difficulties, or the emergence of the parasites seems to coincide completely with the development of the new host scales.

Liberation of *Anicetus ceroplastis*

Our previous report and the decrease in the population of *Ceroplastes rubens* in Kyushu stimulated the liberation and colonization of a Kyushuan race of *Anicetus ceroplastis* by many prefectural entomologists and some of the growers in Honshu and Shikoku. From 1948 to 1951 a number of *Ceroplastes rubens* harboured *Anicetus ceroplastis* were distributed from Kyushu to almost all the prefectures of Honshu and Shikoku where the damage of *Citrus*, *Diospyros* and *Thea* plants by this scale is severe, although it is, as yet, too early to estimate their value as controlling agents in those districts (Fig. 4).

In preparation for transportation, branches and twigs infested with parasitized or non-parasitized scales were cut into 10 to 20 cm. in length and loosely wrapped in paper so as to prevent rubbing. These were then loosely packed in heavy paper cartons

Fig. 4. The internal movement (1948—1951) of *Anicetus ceroplastis*, an Encytid parasite of *Ceroplastes rubens*, following its first liberation to Shizuoka Province in 1948.

or in wooden boxes, sealed, tightly wrapped, securely roped and sent to the stations by registered mail. Upon receiving the material, the twigs or branches were put into the glass vials so to await the emergence of adult parasites. Every day the parasites after emergence were transferred from the vials containing infested twigs to other vials and fed with diluted honey for several days before liberation.

In order to determine the effectiveness of *Anicetus ceroplastis* experimentally in orchards the parasites were introduced into Tsukumi district, Oita Prefecture, Kyushu. About 400 adults of *Anicetus ceroplastis* were liberated in two Citrus orchards in Tsukumi. Both sexes were liberated, but approximately one-half were females.

Colony 1 (Citrus orchard, Citrus Experiment Station).

Colonized 80 adult females on June 20, 1948. A few days after liberation a heavy storm occurred. Not a single adult parasite was recognizable after the storm, and all the parasites seemed to have been killed by the strong wind and heavy rain. Before liberation in 1948 *Anicetus ceroplastis* was not present. In the summer of 1950 I was astonished at the fact that the parasite had successfully established there and could find a number of adult females and males in the orchard. Recolonized in June, 1950, with about 200 females.

Colony 2 (Grower's Citrus orchard).

Colonized with 120 adult females on June 20, 1941. Several parasites were seen even after the heavy storm. Before liberation in 1948 *Anicetus ceroplastis* was not present. During subsequent years the parasites increased steadily. For the two years period of 1948—1949 it parasitized about 13 per cent of *Ceroplastes rubens*.

Thus in no case did the parasite colonies fail to establish themselves.

Effect of Some Insecticides on *Anicetus ceroplastis*

During recent years the problem of the effect of insecticides to the natural enemies has become more and more important. It is common knowledge that insecticides have a detrimental effect on the parasite-predator populations. In 1947 Dr. G. C. Ullyett

classified the adverse effect of insecticides on parasites and predators as follows: a) direct destruction of the parasites or predators by the insecticide; b) destruction of hosts containing developing parasite progeny; c) a repellent effect of the insecticide which keep natural enemies away from the environment of the host; d) decrease in longevity and extent of oviposition by female parasites in sprayed crops; and e) reduction in parasitism and in the increase in predator populations due to decreased host density. Especially the period of newer synthetic organic compounds throw us many difficult problems in the field of biological control. For example the following authors discussed the direct effect of insecticides on parasite-predator population: Annand (1942), Boyce (1950), Clausen (1936), Collins (1934), Cox and Daniel (1935), Cox (1942), Daniel (1935), DeBach (1946, '47), Driggers and Peppers (1936), Ewart and DeBach (1947), Ewing and Evy (1943), Flanders (1942, '43), Gaines (1946), Griffiths and Stearns (1947), Griffiths and Thompson (1947), Griffiths and Fisher (1949), Haug and Peterson (1938), Henderson (1943), Hills (1934), Hodgkiss and Parrot (1914), Holloway and al. (1942), Hough and al. (1945), Hough (1946), Isley (1946), Iyatomi (1949, '51), Jancke (1935), Michelbacher and al. (1946), Myrburgh (1948), Nakayama (1936), Nel (1942), Newcomer and al. (1946), Newson and Smith (1949), Rings and Weaver (1948), Schread and Garman (1934), Smith and Fontenot (1942), Smith and Driggers (1944), Speyer (1936), Summerland and Steiner (1943), Toyoshima (1949), Tsutsui (1949), Walton and Whitehead (1941), Watson (1912), Wheeler and La Plante (1946), Woglum and al. (1947), Yothers and al. (1935), Yothers (1947). And the following cases were reported: HCN gas on *Cryptolaemus montrouzieri* (to control California red scale), tartar emetic on *Comperiella bifasciata* (yellow scale), sulphur dust on *Comperiella bifasciata* (yellow scale), sulphur dust on *Metaphycus helvolus* and *luteolus* (black scale and soft scale), lime-sulphur on parasites (San Jose scale), derris on *Hippodamia convergens*, calcium arsenate on Coccinellidae and Chrysopidae (cotton pests), BHC plus sulphur, Toxaphen plus sulphur or calcium arsenate plus nicotine on *Geocoris punctipes* and *Orius insidiosus* (cotton pests), dormant sprays on parasites (San Jose scale, etc.), kerosene emulsion on parasites (citrus blackfly), arsenical sprays on *Ascogaster carpocapsae* (codling moth), lead arsenate, lead arsenate plus

lime, summer-oil emulsion or nicotine bentonite on *Trichogramma* (codling moth), DDT on *Pseudaphycus* (Comstock mealy bug), Cryolite dust on *Cryptolaemus montrouzieri* (mealy bugs), DDT and rotenone on *Rodolia cardinalis* (cottony cushion scale), sulphur on *Trichogramma* (Oriental fruit moth), DDT and Ryanex on *Macrocentrus ancylivorus* (Oriental fruit moth), DDT and BHC on *Trichogramma japonicum* (rice stem borer, rice green caterpiller), nicotine sulphate, tar distillates, all tar oil preparations, DDT and BHC on *Aphelinus mali* (woolly apple-aphid), DDT on predators (citrus red mite), parathion on parasites (soft scale) DDT on parasites and predators (aphids).

There is little doubt that some insecticidal effects may be expected also in the case of *Anicetus ceroplastis*. In order to illustrate this point it seems necessary to show an example of our schedule for spraying *Citrus* in warmer seasons.

Major pests	Spray	When to apply spray
Phyllocnistis citrella	Nicotine sulphate or pyrethrin plus BHC	From the beginning of June to the end of August. Every other week.
Oxycetonia jucunda	BHC (spray or dust)	May.
Dialeurodes citri	Resin wash	June to August.
Unaspis yanonensis	Lime sulphur plus zinc sulphate or DDT (0.02—0.05%)	May to July.
Pulvinaria aurantii	Resin wash or DDT or BHC (0.05%)	June.
Ceroplastes rubens	Resin wash or DDT (0.02%)	July to August.
Dacus tsuneonis	DDT (0.05%) or BHC (0.04%)	From the end of July to the middle of August. Every other week. Three times.

For the control of *Kakivoria flavofasciata*, a serious pest of persimmon, the use of 2.5% DDT dust (carrier: bentonite or caoline) is said to be very effective.

During the years 1949, 1950 and 1951 studies were conducted with DDT, BHC and some other insecticides against *Anicetus ceroplastis* both in the laboratory and in the field. Of course the

object of these experiments was to determine the adverse effect of insecticides to this parasite. The dosage of insecticides tested in the present investigation was adjusted or followed to our schedule for spraying citrus and persimmon trees. Therefore no effort was made to determine the minimum effective dosage for insecticides against adult *Anicetus ceroplastis*.

Table 4. Effect of various dust upon adult *Anicetus ceroplastis* (♂ ♀) exposed continuously (From 20 to 30 0—24 hours old unfed adults were used in each test) (9. vi. 1949, 21°C).

Dust	Test number	Down in 10 min. Per cent	Down in 2 hour Per cent	Kill in 24 hours Per cent
Bentonite	4	0	100	100
Spore of *Penicillium* sp.	3	0	100	100
DDT (5%)	5	100	100	100
BHC (0.5%)	5	100	100	100
Control	5	0	0	0

Table 5. Results from exposing *Anicetus ceroplastis* (♂ ♀) continuously to dry DDT and BHC residues obtained by evaporating spray mixture in which filter paper of 70 mm. in diameter was dipped. All residues were exposed to weathering for one day (From 20 to 30 0—24 hours old unfed adults were used in each test) (1. vi. 1950, 20°C).

	Test number	Down in 10 min. Per cent	Down in 2 hours Per cent	Kill in 24 hours Per cent
BHC water suspension (0.04%)	5	0	80	100
DDT water suspension (0.05%)	4	0	40	100
DDT water emulsion (0.05%)	4	0	68	100
Control	1	0	0	0

Table 6. Results from exposing *Anicetus ceroplastis* (♂ ♀) continuously to dry DDT and BHC residues obtained by evaporating spray mixture in which filter paper of 70 mm. in diameter was dipped All residues were exposed to weathering for one day (From 20 to 30 0—24 hours old unfed adults were used in each test) (7. vi. 1950, 27°C).

	Test number	Kill in 24 hours Per cent	Kill in 48 hours Per cent
BHC water suspension (0.01%)	2	0	45
DDT water suspension (0.01%)	2	100	100
DDT water emulsion (0.01%)	2	100	100
Control	1	0	0

Table 7. Results from exposing *Anicetus ceroplastis* (♂ ♀) continuously to dry DDT and BHC residues obtained by evaporating spray mixture in which filter paper of 70 mm. in diameter was dipped. All residues were exposed to weathering for one day (From 20 to 30 13 days old adults were used in each test) (5. vii. 1950, 28°C).

	Test number	Down in 10 min. Per cent	Down in 1 hour Per cent	Kill in 3 hours Per cent
BHC water suspension (0.04%)	3	0	87	100
DDT water suspension (0.05%)	3	0	30	80
DDT water emulsion (0.05%)	3	0	74	100
Control	1	0	0	0

Table 8. Residual effect of BHC and DDT upon *Anicetus ceroplastis* (♂ ♀) exposed continuously (From 30 to 40 0—24 hours old unfed adults were used in each test. All residues were exposed to weathering for 10 days) (22. vi. 1949, 20.5°C).

	Test number	Down in 10 min.	Down in 1 hour	Killed in 3 hours	Killed in 5 hours
		Per cent		Per cent	
BHC water suspension (0.04%)	3	0	0	0	0
DDT water suspension (0.05%)	4	0	43	56	100
DDT water emulsion (0.05%)	4	0	75	88	100
Control	1	0	0	0	0

Table 9. Effect of some insecticide sprays upon *Anicetus ceroplastis* (♂ ♀) (From 20 to 30 four days old adults were used in each test) (10. vi. 1949. 22°C and 23. vi. 1951. 23°C).

		Test number	Kill in 6 hours Per cent
Resin wash (alkali 0.40%) Water	1 part 25 parts	2	100
Nicotine sulphate (nicotine 40%) Water	1 part 1000 parts	6	100
Lime sulphur Water	1 part 80 parts	3	100
Lime sulphur Water	1 part 100 parts	3	100
Control (water)		1	0

Parasites sprayed or dusted with DDT usually began to show definite reaction within 5 minutes. Within 6 minutes most of the parasites were down and on their back. Most of the parasites sprayed or dusted with BHC were down and on their back within 20 seconds. In both cases death usually occurred within 2 to 5 hours. On the other hand parasites dusted with bentonite or spore of *Penicillium* sp. crawled about for a long time and struggled to free themselves on their back for more than 10 hours. Death occurred within 24 hours though some lingered as long as 30 hours. Parasites sprayed with resin wash, nicotine sulphate

or lime sulphur and exposed to direct wetting for a few second, dried on blotting paper and held in clean cages lives slightly longer but larger numbers were killed within 5 hours. Dried residues of DDT and BHC either on filter paper or on foliage were decidedly toxic to adult *Anicetus ceroplastis* contacting it. Tables 5—8 show the results obtained for residues containing DDT and BHC that had weathered for periods ranging from one to ten days. The residual effect of DDT lasted more than three weeks, while that of BHC lasted only for a few days. This toxicity was noted under field conditions by observing parasites that visited sprayed foliage. Examination shows that most of the lethal effects take place in the first 2 hours and is completed within 24 hours. About ten to twenty minutes exposure to DDT residue represented, in the present tests, on the parasites also a catastrophe. Results obtained from field spraying of resin wash, nicotine sulphate or lime sulphur were not quite the same as in the laboratory conditions since spraying usually disturbed the parasites and some flew away to avoid the spray. So far as my experiments go, foliage or branches which were sprayed with resin wash, nicotine sulphate or lime sulphur and dried had little or no effect on adult *Anicetus ceroplastis* which were exposed to the surface. From the experiments performed it became clear that insecticides have a detrimental effect on the production of *Anicetus ceroplastis* by repelling the host-searching females or by the destruction of the adult parasites and the application of insecticides for the control of other pests should be so timed that parasites of the scales are least affected. As mentioned before, parasites, *Anicetus ceroplastis*, thrive best when the scale is in all stages of development especially in early summer season and anything, for example spraying or dusting, that brings about an even-hatch condition of the scale has adverse effect upon the parasites, since existence of the scales of all stages of development (except for the youngest and full-grown individuals) is needed for the effective oviposition of the parasites. In this connection Dr. Flanders (1942) showed that in the case of *Metaphycus helvolus* (Compere) "insecticides tend to decrease the effectiveness of this parasite indirectly by bringing about an even-hatch condition of the host generations so that there is a lack of food for the parasites."

Next it is worthy of consideration that the elimination of one major pest by some insecticides has sometimes left the trees open to attack by another that may have been controlled by it. Dr. Ullyett (1947) discussed the problem on the increase of the associated pests by the elimination of major pests and wrote as follows:—"In many instances, the use of insecticides for the control of one insect pest of a crop has resulted in another pest, which may be normally of little importance, assuming epidemic proportions. Scale-control problems become more difficult each year in California and that a similar tendency is apparent in other countries." Such problems have been treated by the following authors—Gardner (1934), Gilliatt (1935), Driggers and Pepper (1936), Boyce (1936, '50), Osburn and Spencer (1938), Schoene (1938), Steiner (1938), Ingram and al. (1947), DeBach (1947), Ewart and DeBach (1947), Carter (1949), etc. For example Boyce (1950) wrote:—"Almost from the beginning of the use of DDT on agricultural crops, in 1944, 'side problems' resulting from an upset in the balance of certain parasite-host or predator-host relationships have been apparent. This generally striking effect has greatly stimulated interest in this question. Upsets in the natural balance which have been observed on Citrus in California from the use of DDT involve the following pests: cottony-cushion scale, citrus red mite, citrus mealybug and other mealybugs, California red scale, yellow scale, orange Tortrix, and several species of aphids; from use of sulphur: black scale and the citrus mealybug; and from the use of parathion: soft (brown) scale." Further the following example exhibited by Dr. Carter (1949) is of extreme interest:—"Dr. Carter exhibited a mango leaf heavily infested by this wax scale (*Ceroplastes rubens*). The tree had been given heavy applications of DDT about a year ago in an effort to combat *Dacus dorsalis*. The scale infestation on this particular tree is in striking contrast to untreated trees nearby which are relatively free from the scale." In Hawaii, *Ceroplastes rubens* has been known as a minor pest and this fact is apparently responsible to the activity of three Hymenopterous parasites, viz. *Anerisitus ceroplastae* Howard, *Microterys kotinskyi* (Fullaway) and *Tomocera californica* Howard. In Japan the following close relationships exist among insect pests of major importance and their natural enemies in citrus orchard.

Major pests	Natural enemies
Unaspis yanonensis (Kawana)	*Chilocorus kuwanae* Silvestri
Aonidiella aurantii Maskell	*Comperiella bifasciata* Howard
Pulvinaria aurantii Cockerell	*Chilocorus kuwanae* Silvestri *Chilocorus rubidus* Hope
Ceroplastes rubens Maskell	*Anicetus ceroplastis* Ishii
Icerya purchasi Maskell	*Rodolia cardinalis* Mulsant
Aleurocanthus spiniferus Quaintance	*Prospaltella smithii* Silvestri

In Tsukumi District spraying of DDT or BHC has been recommended in recent years to control *Dacus tsuneonis* Miyake, a local major pest, or to prevent the oviposition of this fly in citrus orchard. The spraying is applied three times (every other week) from the end of July to the middle of August. Thus the residual effect of DDT may last until the beginning or middle of September, and not only a number of natural enemies listed above will be killed during this period but also many of the scales of major importance will increase prominently. Therefore the application of insecticides, especially those having residual effect, on trees infested with scales would be undesirable or must be so timed as to save the natural enemies until the parasites and predators establish themselves and natural control can become effective in citrus or some other orchards.

Some Practical Suggestions on the Liberation or Colonization of the Key Parasite

Until we establish a method of successful mass production of both *Ceroplastes rubens* and *Anicetus ceroplastis*, it is desirable to collect twigs of various plants, such as *Fortunella* sp., *Citrus Unshu*, *Laurus nobilis*, *Ilex Oldhami*, *Ilex integra*, *Ilex latifolia*, *Eurya japonica montana*, etc., which are infested with *Ceroplastes rubens* in Kyushu, so as to emerge as many adult *Anicetus ceroplastis* as possible and then release only the parasites to any desired places in Honshu, Shikoku and Kyushu where this scale insect has been a serious pest for the past forty to fifty years. In this connection the liberation or colonization of a large number of parasites is of course the first essential. As mentioned before the scales harbour full-grown larvae and pupae of the parasite in

April or May. Therefore we may be able to expect almost 100 per cent emergence of the parasites from the scales collected in the middle or later part of the spring season. If we collect the twigs infested with scales in August or September, some parasite progeny may fail to complete the larval life and cannot reach the adult stage, since the parasites within the scales are in all stages of development in August or September. Consequently we shall run a risk of destroying some progeny of the parasite if we collect the twigs in Kyushu in the early part of the autumn. Next it is important to select the twigs infested with scales of comparatively small size (average length of the longitudinal axis of the wax covering being 2.5 mm. or less). The reason is that the scales parasitized by parasites in the autumn season cannot make normal growth as in sound scales. Thus scarcely any parasite may emerge from the scales of much larger size in the spring season.

As indicated before, orchard parasitization may be depressed following regular and thorough application of insecticides. Therefore, it must be emphasized that the orchard or the plants where parasites are liberated must be left unfumigated or unsprayed for a period of at least two or more years, irrespective of the existing scale populations, or the growers wishing to enjoy the advantage of natural control must actually encourage a certain degree of scale infestation over a period sufficient to enable the parasites to establish populations which take a position to control the pest in the future. On the other hand establishments of the parasites could best be done by liberating the parasites in non-commercial orchards or on some non-commercial plants near or surrounding the commercial orchards. Heavy infestation of plants by *Ceroplastes rubens* growing in other places than orchards may be responsible for the production of a large number of parasites. The parasites would thus become plentiful after parasitizing the scales and be ready to attack the scales of the commercial orchards. This method would well correspond with the principle of the so-called strip farming or croping. In addition to the above suggestion I am firmly convinced that in most, if not all, of the places where *Ceroplastes rubens* is making severe damage, the climatic condition is also favourable to *Anicetus ceroplastis*.

REVISED LIST OF THE HYMENOPTEROUS PARASITES OF *Ceroplastes rubens* MASKELL

Parasites	Localities	References
Anabrolepis bifasciata Ishii	Japan	Yasumatsu et Tachikawa (1949)
Anabrolepis extranea Timberlake	Japan	Yasumatsu et Tachikawa (1949)
Aneristus ceroplastae Howard	Hawaii	Timberlake (1918), Fullaway (1919)
	Fiji	Simmonds (1936)
Anicetus ceroplastis Ishii	Japan	Yasumatsu et Tachikawa (1949), Yasumatsu (corrected in this report)
Aphytis sp.	Japan	Yasumatsu et Tachikawa (1949)
Casca sp.	Japan	Yasumatsu et Tachikawa (1949)
Cerapteroceroides japonicus Ashmead	Japan	Ishii (1940)
Cheiloneurus ceroplastis Ishii	Japan	Ishii (1923, 1928, 1932, 1940), Kaburaki (1934)
Coccophagus hawaiiensis Timberlake	Japan	Ishii (1923, 1932, 1940), Kaburaki (1940), Yasumatsu et Tachikawa (1949)
Eupelmus sp.	Japan	Ishii (1932)
Eusemion sp.	China	Silvestri (1929)
Marietta sp.	Japan	Ishii (1940)
Metacerapterocerus fortunatus (Ishii)	Japan	Original record *
Microterys kotinskyi (Howard)	Hawaii	Fullaway (1918)
Microterys okitsuensis Compere	Japan	Yasumatsu et Tachikawa (1949)
Microterys speciosus Ishii	Japan	Ishii (1923, 1928, 1932, 1940), Kaburaki (1934), Yasumatsu et Tachikawa (1949)
Physcus atrithorax Girault	Australia	Girault (1939)
Quaylea whitieri (Girault)	Hawaii	Ishii (1940)
Tomocera californica Howard	Hawaii	Fullaway (1919), Smith et Compere (1928)
Tomocera ceroplastis Perkins	Hawaii	Fullaway (1919)

* 1♀, Kochi, Shikoku, 8. vi, 1950, reared by Mr. T. Yoshii, ex *Ceroplastes rubens*.

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REVISIONAL NOTES ON *CAMPONOTUS HERCULEANUS* LINNÉ AND CLOSE RELATIVES IN PALEARCTIC REGIONS (HYMENOPTERA : FORMICIDAE)

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The familiar black and red-and-black carpenter ants related to *Camponotus herculeanus* Linné form an essentially Holarctic group of a few species and subspecies bearing a great superfluity of names. For convenience only, the group may be divided geographically into Eurasian and North American sections. The North American forms have recently been treated fully by Creighton (1950), and his work is accepted with two alterations (Brown, 1950).

Relatively little synonymy applies among European forms, although the identity of Linne's original *C. herculeanus* may be questioned. The chief problem here is the separation of the "accepted" *herculeanus* from the closely related *ligniperda* (Latreille); certainly the two species have been confused by many European myrmecographers. Menozzi (1922) has attempted to put their separation on a more definite basis by claiming differences in the male genitalia in addition to the more commonly accepted criteria of color and sculpture in the female and worker castes. Our examination of the genitalia in a few specimens of each form has failed to show significant genitalic differences, but more material would perhaps be more revelatory of characters. It seems probable that *ligniperda* exists as a separate specific entity, however, though its ethological, distributional and even morphological features are in need of clarification by some specialist with a *broad* field knowledge of both common forms.

Emery has rightly eliminated Forel's variety *herculeano-ligniperda* as representing the small, dark minim workers of the "typical"

ligniperda. Menozzi's variety *nadigi* (1922) is an almost completely black form of *herculeanus* such as is found at many other points in the range of the latter. The present evidence does not support its treatment as a geographical race, and we provisionally consider it a synonym. The European fauna is considered to consist of *C. herculeanus*, *C. ligniperda* and *C. vagus* Scopoli. The lastnamed is quite distinct as a species, and need not be mentioned further here.

The Asian fauna of the group has been dealt with successively and almost completely intra-nationally by western European, Russian, American and Japanese specialists. It is scarcely surprising that there exist one or more each European, Russian, American and Japanese names for most forms which have been considered distinct even in the past. The terrible taxonomic tangle resulting from this nomenclatorial short-sightedness should serve as a warning to taxonomists who refuse to study variation beyond the nearest political boundaries. It is the chief purpose of the present paper to analyze the Asian members of the group on the basis of modern population systematics and to unravel the nomenclatorial tangle as far as possible. The pertinent literature is so widely scattered and voluminous that it is almost certain we have overlooked many references, especially in the more recent European and Russian journals. While many of these may contain valuable distributional or ethological data, the probability of misidentifications occurring in them is strong enough to render their uncritical acceptance only a further source of confusion.

In order that the reader may follow our discussion more easily, we here present our systematic conclusions regarding the Eurasian fauna in skeleton arrangement:

Camponotus herculeanus herculeanus (Linné)
=var. *nadigi* Menozzi (new syn.)
=var. *montana* Ruzsky (Emery syn.)
=var. *whymperi* Forel (Creighton syn.)
=var. *jacutica* Karawajew (new syn.)

Camponotus herculeanus japonicus Mayr
(a) Absolute synonyms
=var. *miltotus* Wheeler (new syn.)
=subsp. *wui* Wheeler (new syn.)

(b) Concolorous or nearly concolorous black forms referable mainly to *japonicus*, but with clypeus and pubescence variably tending toward those of *C. h. herculeanus*. Intergrades, *japonicus* × *herculeanus*.

=var. *aterrima* Emery (new syn.)
=var. *sachalinensis* Forel (new syn.)
=var. *saxatilis* Ruzsky (new syn.)
=var. *manczshurica* Ruzsky (new syn.)
=var. *sanguinea* Karawajew (new syn.)

c) Forms with pubescence of *japonicus*, clypeus intermediate, head and gaster black, alitrunk red. Intergrades, *japonicus* × *herculeanus*.

=var. *cruentata* Karawajew (nom. praeocc.)
=var. *atrox* Emery, nom. pro. *cruentata* (new syn.)
=var. *koreanus* Teranishi (new syn.)
=var. *jeholensis* Teranishi (new syn.)

Camponotus ligniperda (Latreille)
=var. *herculeano-ligniperdia* Forel (Emery syn.)
Camponotus obscuripes obscuripes Mayr
Camponotus obscuripes hemichlaena Yasumatsu et Brown, subsp. nov.
Camponotus yessensis Teranishi (raised to species)
Camponotus formosensis Wheeler (raised to species)
Camponotus vagus Scopoli (confirmed as a good species)

Species inquirendae

Camponotus punctatissimus Emery
Camponotus cilicicus Emery
Camponotus vagus var. *kodorica* Forel
Camponotus herculeanus eudokiae Ruzsky

The Relationship of *Camponotus herculeanus* to *C. japonicus*

The chief characters of taxonomic value in the *herculeanus* complex are pilosity and color; density and opacity of gastric sculpture and the slightly advanced clypeal lobe of *japonicus* are of importance also. *C. ligniperda* has the most shining gastric surface, with very light cross-striation, and usually has the entire alitrunk as well as a considerable portion of the first gastric segment red. Its gastric *pubescence* falls far short of reaching the

posterior borders of the segments in the middle. *C. herculeanus* has somewhat stronger gastric sculpture; the body is most often black except for the legs and a small area containing the posterior propodeum, node and sometimes the extreme base of the gaster, which are deep red; the gastric pubescence usually reaches nearly to the posterior borders of the gastric segments in the middle and only rarely slightly surpasses them (the latter is probably and intergradient condition). *C. japonicus* has quite the heaviest gastric sculpture, and this produces a silky opacity of the surface; the body and legs are typically concolorous black; the gastric pubescence is yellowish, coarse and dense, surpassing the posterior borders of the segments often by half or more of its length. *Ligniperda* will not concern us further here.

In dealing with color, it must be emphasized that the complex varies considerably according to *ecological* as well as *genetic* factors. This is especially true of *herculeanus* and *japonicus*. *C. herculeanus herculeanus* normally nests in timber in cold boreal or subalpine regions. Near timberline on Mt. Washington, in the northeastern United States, the only trees (*Picea*) are strongly dwarfed and not suitable for nesting, and *herculeanus* is forced to nest in the thin soil beneath rocks under extremely cold, arid and exposed conditions. Nests taken by Brown in this locality are all "depauperate" in that the workers are all smaller and much lighter in color than in "normal" nests from lower altitudes on the same mountain. The timberline workers have the alitrunk a faded red throughout.

A somewhat similar phenomenon may be noted in *japonicus*. Specimens from arid regions in North China often have large parts of the head dark red (var. *miltotus* Wheeler). Brown has taken workers, usually majors, of this type in Shensi running in the same files with "normal" black specimens. Where cold and aridity reach extremes together, as in the high mountains of Sikang, both D. C. Graham and Brown found nests with all or most of the workers having almost the entire head and alitrunk a pale reddish color. It is therefore evident that color variants in this group must be treated with the utmost caution.

In Asia, *herculeanus* is most typically associated with the boreal and alpine coniferous forests of the Siberian taiga and the highest mountains of China, Japan, trans-Himalaya, etc.

Japonicus is typically a soil nester in the more temperate plains and lower mountains of China and Japan. The ranges of the two reach toward each other and are deeply interlocked in the mountain chains so abundant in eastern Asia. The present evidence shows, we believe, that the two forms can and do intergrade in most or all intermediate zones, although the transition appears fairly sharp in mountainous regions. This sharp transition is probably due in large part to the activity of man in lumbering and clearing the middle mountain slopes for agriculture, a practice followed widely in China and Japan. The relatively undisturbed regions are remote, but would repay study.

Intergradation of a much more gradual sort is fairly clear in a review of low-altitude material ranging from south to north in East Asia. The slightly projecting clypeal border and dense long pubescence which characterize *japonicus* become less and less marked as one goes northward in China and Manchuria, producing the forms known as *aterrima*, *sachalinensis*, *saxatilis* (Ural Mts.), *manczshurica* and *sanguinea*, which seem to grade insensibly into *herculeanus*. This intergradation occurs over a wide belt stretching from the southern Urals to Sakhalin. Unfortunately, we have little or no information concerning the state of affairs in wide stretches of Central Asia: Sinkiang, Chinghai, Mongolia; if our interpretation is correct, intergrades should occur in these regions on the isolated mountain ranges.

The forms called *atrox*, *jeholensis* and *koreanus* are approximately equivalent and are essentially *japonicus* in sculpture and pilosity, while the alitrunk is a rather uniform red, a color type commonly encountered among *herculeanus* populations (see above). This form has been reported (in the original descriptions) from mountains in northern Korea and Manchuria, and Yasumatsu has taken specimens in the mountains of Shansi.

The forms discussed in the two foregoing paragraphs are considered by us to represent the array of phenotypes expected of a hybrid swarm formed at the meeting of two geographical races which have been or less completely separated, possibly during the relatively mild glaciation undergone by East Asia. This swarm appears to follow conditions mentioned in Ernst Mayr's "Evolution and the Origin of Species" (1942). It is interesting to note that *japonicus* specimens with slightly thinned gastric

pubescence from Boketu and White River, Barum, Manchuria (P. and H. Dorsett) in the Museum of Comparative Zoology, which were determined as "*aterrima*" by Wheeler, are accompanied by a collector's note stating that they were taken from the bases of willow trees. Does this indicate an intergradation in nesting habits, as might be expected to occur?

Carrying the data to their logical conclusion, we here treat *japonicus* as a southern geographical race of *herculeanus* in spite of the fact that the great majority of specimens we have seen are definitely assignable to one or the other name. We place the intergrades in the synonymy of *japonicus* because most of them seem closer to the southern race, but some of them could equally well be placed under the "typical" *herculeanus.* Illustrative of the confusion is an extract of a letter from the late Dr. Karawajew to Teranishi, both of whom worked extensively in the complex:

". . . . *jacutica* is a synonym of *sachalinensis*; *aterrima* Teranishi (Zool. Mag., *41*: 240)=*saxatilis*; *aterrima* Karawajew (Rev. Russ. d'Ent., *12*: 594, 1912)=*jacutica*."

The form *jacutica*, of course, is the "typical" *herculeanus*, which in Asia has gone under the names *whymperi* and *montana* as well. Specimens of *herculeanus* with "typical" pilosity and sculpture, but entirely black, are known from the highest Japanese mountains, and we have seen similar specimens from near the top of the small island Uotsurijima (=Hoa Pin San) at about 1200 feet altitude. This island is only a short distance off the northern end of Formosa. The specimens were furnished by the collector, the late Mr. Masaki; they probably represent an isolated colony originated by a wind-borne female. It is an interesting corollary of this arrangement that *herculeanus* seems to produce extreme melanic forms in the southern montane extensions of its range on all continents; only in North America does the melanic population seem to show the characteristics of a reasonably well-defined geographical race in *C. herculeanus modoc* Wheeler. Further, more intensive studies of this group in such montane regions may show that the situation is much more complex than our arrangement admits at present. We have gone as far here as purely morphological and distributional data will permit, and we believe that future students will have to resort to interbreeding and trans-

plantation experiments in order further to clarify the relationships between the populations.

Camponotus herculeanus herculeanus (Linné)

Formica herculeana Linné, 1758, Syst. Nat., edition 10, **1**: 579.

Camponotus herculeanus Emery, 1925, Gen. Ins., Fasc. 183, pp. 72 73. See for further synonymy and older references.

Camponotus herculeanus pennsylvanicus var. *whymperi* Forel, 1902, Trans. Ent. Soc. London, p. 669, ♀♀, original description from N. American specimens. Emery, 1925, loc. cit., synonymy. Forel, 1903, Ann. Mus. Zool. Acad. Imp. Sci. St. Petersbourg, 8: 14, Siberie de sud-ouest. Kisselewa, 1925, Ber. Tomsker Staats-Univ., **75**: 73, Ussuri. Ruzsky, 1936, Trav. Inst. Sci. Biol. Tomsk, **2**: 89 90, Transbaikalgebiet.

Camponotus herculeanus Kisselewa, 1925, Ber. Tomsker Staats-Univ., **75**: 75, Ussuri. Ruzsky, 1936, Trav. Inst. Sci. Biol. Tomsk, **2**: 89, Transbaikalgebiet. Creighton, 1950, Bull. Mus. Comp. Zool., Harvard Univ., **104**: pp. 363 370, discussion of *C. herculeanus* group in N. America and synonymization of var. *whymperi*. See also European records and other information in numerous faunal lists and other papers by European and Russian writers, esp. Kuznezov-Ugamsky, Karawajew, Holgersen, et al. For biology, see Eidmann, esp. 1928, Zeitschr. f. angewandte Ent., pp. 229-253, 9 figs.

Camponotus herculeanus var. *jakutica* Karawajew, 1929, Mem. Classe Sci. Phys. Math. Acad. Sci. Ukraine, **8**: 210, original description, Yakutsk. 1931, Zool. Anz., **93**: 30, Yakutsk, Ussuri, Irkutsk, Umgebung des Baikalsees, Sakhalin. 1931, Ibid., **94**: 107, Irkutsk, Sakhalin, Yakutsk, (new synonymy).

Camponotus herculeanus herculeanus Kôno et Sugihara, 1939, Trans. Kansai Ent. Soc., **8**: 10, Sakhalin, northeastern Hokkaido. (For additional *partim* synonymy see references under var. *sachalinensis* given in the synonymy of *C. herculeanus japonicus* below. Probably most *sachalinensis* records are based on the black, near-typical form of *herculeanus*, but so long as these forms are considered intergradient between the two races it matters little under which name they are placed in synonymy.).

Distribution: *C. herculeanus herculeanus* has a Holarctic range surpassed by few other ants. It is found almost everywhere within the natural ranges of the coniferous genera *Picea* and *Abies*, particularly the former, the presence of which seems almost to be a prerequisite to the development of the ant in its most typical form. This attachment is more or less exclusive, for other species of *Camponotus* are rare or absent in the various muskegs, taiga and montane forests of the spruce belt. The fact that spruce and spruce-fir forests are usually relatively discontinuous may provide one reason for the seeming scarcity of intergrades between "typical" *herculeanus* and *japonicus* in mountain areas in Japan and China south of Manchuria. This statement of ecological

preference is based on rather scanty data and on inferences drawn from locality reports, as well as from limited observations by the junior author in North America. It is unfortunate that the great majority of published records contain little or no ecological data.

Camponotus herculeanus japonicus Mayr

Camponotus japonicus Mayr, 1866, Verh. Zool.-bot. Ges. Wien, **16**: 885, ♀, original description, Japan.

Camponotus japonicus Emery, 1925, Gen. Ins., Fasc. 183, pp. 72-73, further references.

(a) *absolute synonyms*

Camponotus japonicus var. *miltotus* Wheeler, 1929, Amer. Mus. Novit., No. 361, p. 9, ♀ major, (new synonymy).

Camponotus japonicus subsp. *wui* Wheeler, 1929, Ibid., p. 9, ♀ media, (new synonymy).

(b) *Black intergrades to herculeanus*

Camponotus japonicus var. *aterrima* Emery, 1894, Ann. Mus. Stor. Nat. Genova, **34**: 478, nota, ♀ ♀, original description as *C. pennsylvanicus* var. (new synonymy). Emery, 1925, Gen. Ins., Fasc. 183, p. 73, further references. Karawajew, 1913, Rev. Russe d'Ent., **12**: 592, part.? Amur, Korea, Sakhalin. 1927, Trav. Mus. Zool. Acad. Sci. Ukraine, **2**: 344, Ussuri, Irkutsk. Mocsáry, 1901, Dritte Asia. Forschungsreise Gr. E. Zichy, **2**: 132, "China": Daba, Kalgan, Tshan-pin-cho. Baltz, 1915, Rev. Russe d'Ent., **25**: 318, observation. Kisselewa, 1925, Ber. Tomsker Staats-Univ., **75**: 73, Ussuri. Santschi, 1925, Bull. Soc. Vaud. Sci. Nat., **56**: 88, Quelpart I. Wheeler, 1931, Peking Nat. Hist. Bull., **5**: 75, summary of records from China, incl. records published by Gee and by Wheeler 1919–1929. Ruzsky, 1915, Ann. Mus. Zool. Acad. Sci. Petersbourg, **19**: 479, Tibet. 1936, Trav. Inst. Sci. Biol. Tomsk, **2**: 90, Transbaikalgebiet. Teranishi, 1929, Zool. Mag., Tokyo, **41**: 240, E. Siberia, China, Korea, Sakhalin, Hokkaido. 1936, Insects of Jehol, VII. Formicidae, pp. 3, 10, fig., Jehol. 1940, Posthumous section in Teranishi Memorial Volume, p. 72, southern Sakhalin, central Hokkaido. Sjöstedt, 1935, Arkiv f. Zool., **28** *A* : 5, Kamchatka [?].

Camponotus herculeanus var. *sachalinensis* Forel, 1904, Ann. Mus. Zool. Acad. Sci. Petersbourg, **8** : 281, original description, Sakhalin, Mongolia, Manchuria, (new synonymy). Emery, 1925, Gen. Ins., Fasc. 183, p. 73, further references. [often referred to *C. h. pennsylvanicus* as a var.] Yano, 1910, Zool. Mag., Tokyo, **22**: 422, Sakhalin, Siberia. Karawajew, 1913, Rev. Russe d'Ent., **12**: 592, Baikal, Manchuria. 1927, Trav. Mus. Zool. Acad. Sci. Ukraine, **2**: 344, Ussuri. Teranishi, 1932, Trans. Kansai Ent. Soc., **3**: 50, Sakhalin, Shinshu district of Honshu-2000-2300 M. alt. Uchida, 1936, Biogeographica, Tokyo, **1**: 72, Mt. Daisetsu in central Hokkaido; rarer than *saxatilis*. Morishita, 1945, Nippon Seibutsushi, **5**: Insecta, **2**: 17, 21–23, 27, distribution in central mountainous regions of Japan; lower limit of vertical distribution: 1300 M.

Camponotus herculeanus saxatilis Ruzsky, 1895, Trav. Soc. Nat. Univ. Kasan, **28** (5): 7, original description, all castes. (new synonymy) Emery, 1925, Gen. Ins., Fasc. 183, p. 73, as var. of *C. h. pennsylvanicus*, further references, southern Ural Mts. to Volga R.

Camponotus japonicus var. *manczshurica* Ruzsky, 1915, Ann. Mus. Zool. Acad. Sci. Petrograd, **19**: 481, ♀, Manchuria, (new synonymy).

Camponotus japonicus var. *sanguinea* Karawajew, 1929, Mem. Acad. Sci. Ukraine, **13**: 212, all female castes, (new synonymy).

(c) *Intergrades to herculeanus with red alitrunk*

Camponotus herculeanus Japonicus var. *cruentata* Karawajew, 1912, Rev. Russe d'Ent., 12: 595, ♀, nec Latreille; original description, Tshinjasi-san, N. Korea, (preoccupied).

Camponotus japonicus var. *atrox* Emery, 1925, Gen. Ins., Fasc. 183, p. 73, pro *cruentata* Karawajew, nec Latreille, (new synonymy).

Camponotus herculeanus japonicus var. *cruentata* Teranishi, 1929, Zool. Mag., Tokyo, **41**: 241, N. Korea, Mt. Kongo in central Korea.

Camponotus (*C.*) *herculeanus jeholensis* Teranishi, 1936, Insects of Jehol, VII. Formicidae, pp. 4, 10, fig., Jehol, (new synonymy).

Camponotus (*C.*) *herculeanus* [sic] var. *koreanus* Teranishi, 1940, Posthumous section in Teranishi Memorial Volume, p. 71, Mt. Kongo in central Korea, (new synonymy).

Examples from the southern Urals (*saxatilis*) and many from North China, Manchuria, etc. are so similar to the "typical" *japonicus* from Japan that they cannot really be separated, so that *saxatilis* and *aterrima* might be considered as intergrades or as absolute synonyms with equal justice. The description of *sanguinea* was published in the same year as that of *miltotus*, and Karawajew obviously had a form agreeing closely with Wheeler's type in the Museum of Comparative Zoology. The form Wheeler called *wui* clearly belongs to *japonicus*; the yellowish borders and other features of the gaster are found in all *japonicus* if examined closely, especially crushed specimens. We have not seen *manczshurica*, but the description answers well to intergradient specimens from Manchuria. If future study were to show that *japonica* is really a distinct species, all the above forms would probably still have to be considered synonymous with it. *Sachalinensis*, as already mentioned, seems truly intergradient, and most of the specimens on which the literature is based could be put under *herculeanus herculeanus* more easily than under *japonicus*.

The forms *atrox* (*cruentata*), *koreanus*, and *jeholensis* appear to be equivalent. It is doubtful that the name *koreanus* would have seen print had Teranichi lived longer. This color variant of *japonicus* is widely but discontinuously distributed in the

mountains of Korea, Manchuria and North China; Yasumatsu has taken it in the mountains of Shansi. If *japonicus* is ever returned to specific status, "*atrox*" may well have to be considered as an alpine subspecies.

The subspecies *japonicus* is probably the most conspicuous and familiar of all ants throughout the plains and lower hill regions of China and much of Japan. Emery has recorded it from upper Burma. Brown has seen specimens from D. C. Graham and has collected specimens himself from western China at altitudes up to nearly 2500 meters in the mountains along the Szechuan-Sikang (Chinese Tibet) border; in Shensi, *japonicus* was plentiful at 2100 meters in the Tsin Ling Shan at Miao T'ai Tze. The nests are built in the soil, commonly along the dikes between the rice-paddies, and are often furnished with a disorderly crater of coarse earth particles. The nest entrance may be up to a centimeter in diameter. The southern limits of the distribution are unknown, but since the ant is common at Kunming, it must extend down the mountains of Burma and Indo-China for some distance. It has not been taken by any of the recent collectors in Formosa or Okinawa, nor is it known just how nearly continuous the range across to the Volga is from Mongolia. The similarity to *Camponotus pennsylvanicus* of the eastern half of North American is particularly striking.

While we are forced by the available data to consider *japonicus* as most likely a subspecies of *herculeanus*, we so assign it with great reluctance and some uneasiness, and we by no means consider the question completely closed. We believe, however, that the subsidiary synonymy suggested above is for the most part firmly founded. The removal of these doubtful names from the taxonomic field is necessary to the development of the knowledge of the group and is long overdue.

Camponotus obscuripes obscuripes Mayr

Camponotus ligniperdus var. *obscuripes* Mayr, 1871. Verh. Zool.-bot. Ges. Wien, **28**: 645, ♀, original description.

Camponotus ligniperdus (nec Latreille) F. Smith, 1874, Trans. Ent. Soc. London, p. 402, Japan: specimens were from Hiogo. Matsumura, 1911, 1930, 1931, 1932, in various editions and modifications of Illustr. Ins. Japan, with figures, partim. Of localities cited, *obscuripes* does not occur in Kyushu, Korea, China or Europe.

Camponotus ligniperdus var. *obscuripes* Forel, 1901, Mitt. Naturh. Mus., Hamburg, **18**: 70, Yesso [now called Hokkaido]. André, 1903, Bull. Mus. Hist. Nat. Paris.

p. 128, Tokyo. Matsumura, 1911, Jour. Coll. Agr., Tohoku Imp. Univ., **4**: 99, southern Sakhalin.

Camponotus herculeanus obscuripes Emery, 1908, Deutsche Ent. Zeitschr., p. 185, ♀. Watanabe, 1935, Fauna of Towada and Hakkoda Districts, p. 44, Mt. Hakkoda and Tsuta, Honshu. 1937, Cat. Injur. For. Ins. Japan, p. 4, injuring timber. Yasumatsu, 1938, Ins. Japon. Illustr. Icon. Color. Nat. depicta, p. 350, pl. 157, fig. 614, ♀ ♀, partim, short description, Sakhalin to Shikoku, Kyushu incorrect. Konô et Sugihara, 1939, Trans. Kansai Ent. Soc., **8**: 9, Hokkaido.

Camponotus (s. str.) *ligniperda* var. *obscuripes* Yasumatsu, 1940, Mushi, **13**: 1, Tsushima.

Camponotus ligniperdus obscuripes Forel, 1907, Mitt. Natur. Mus. Hamburg, **24**: 19, Gefu, recte Gifu.

Camponotus herculeanus var. *obscuripes* Masaki, 1937, Kontyû, **11**: 84, Hachijo I. Yuki, 1938, Hiroshima Konchu Dokokaishi, **4**: 12, Itsukushima. Nakano, 1938, Kansai Konchu Zasshi, **5**: 83, Sakhalin.

Camponotus herculeanus ligniperda var. *obscuripes* (some references with slight variations in spelling) Yano, 1910, Zool. Mag., Tokyo, **22**: 422, partim, Hokkaido and Honshu only. Wheeler, 1928, Boll. Lab. Zool. Portici, **21**: 117, 127, partim, Honshu only. Teranishi, 1929, Zool. Mag., Tokyo, **41**: 239–240, partim, Hokkaido, Honshu, Shikoku only; alates captured: 6/iv Kyoto, 7/v Mt. Chikuba, 30/v Gumma Prefecture, 8/vii Kamikochi, 25/v Sapporo; nests at roots of pines. Imanishi, 1930, Kontyû, **4**: 186, Mt. Tateyama, Honshu. Teranishi, 1931, Trans. Kansai Ent. Soc., **2**: 28, Shikotan, Kurile Is. 1940, Teranishi, in Posthumous Section of Teranishi Memorial Volume, p. 71, partim, Hondo and Shikoku, non Kyushu, Korea or Saishu I. Sugihara, 1933, Kansai Konchu Zasshi, **1**: 79, Shikoku, alt. 800–1600 M. Baba, 1935, Mushi, **8**: 25, Sado I. Baba, 1946, Mag. Ins. Ecology, **1**: 27, Musashi Prov. 1947, Ibid., **2**: 18, fig., ♀, Mt. Ontake, Honshu, struggle with *C. kiusiuensis* Santschi. Yoshioka, 1939, Trans. Kansai Ent. Soc., **8**: 67, Kiryu, Gumma Prefecture, Honshu. Morishita, 1945, Mushi, **13**: 22, 25, southern Hokkaido, northern Honshu. Azuma, 1938, Ent. World, **6**; 242, Minoo, Honshu. 1950, Ent Rev. Japan, **5**: 47–48, Pl. 1, fig. 3, ♀, further references.

In the synonymy above, we have tried to give a complete summary of references, particularly those appearing in Japanese journals. The reader should note that all previous records from Kyushu and extreme southwestern Honshu belong to the subspecies *hemichlaena* nov. The typical *obscuripes* ranges from the mountains of Shikoku north to southern Sakhalin and the southern Kuriles; in the west, its range extends to Tsushima in the Strait. The Korean record refers to the form "*atrox*" of *japonicus*, and records of *ligniperdus* or *obscuripes* from Quelpart Island are probably erroneous also.

In color, *obscuripes* usually differs in that the red of the alitrunk and gastric base is lighter and more yellowish than in European *ligniperda*; however, some Japanese specimens are equally dark. Many Japanese specimens show very feeble bluish

metallic reflections on the integument, not seen in the European *ligniperda*. The sculpture and pubescence of the gaster are very similar in the two species, and the prime difference still used is the blackish color of the legs in *obscuripes*. While this is a relatively minor difference, we believe that distributional characteristics force the specific recognition of the Japanese form. Apparently, the true *ligniperda* does not occur in East Asia; at least, it has not been reported in the literature available to us. It is surprising that neither form seems to occur in China or Korea, but our study certainly indicates that this is the case.

Camponotus obscuripes hemichlaena subspecies nov.

This subspecies has been included in many former reports of *obscuripes*. For partim synonymy, refer to *C. obscuripes obscuripes*; all previous records from Kyushu and probably some from Honshu refer to *hemichlaena*. Investigation during 1950 indicates that very rarely the typical *obscuripes* occurs in the mountains of Kyushu (Shiiba and Mt. Unzen). We have not yet found *obscuripes obscuripes* individuals in *hemichlaena* nests, or vice versa. The Kyushu populations of typical *obscuripes* may either represent alpine intrusions from the north, or else may be relicts of an original and uniformly typical *obscuripes* population dating from glacial times. The situation with regard to intergrades not being clear as yet, it is impossible to determine with complete finality whether *hemichlaena* is a good species, a geographical race, or possibly even a mere ecotype. Since we have scant reason to consider the black prothorax as other than a well fixed genetic character, and taking into account the distributional evidence, we feel that *hemichlaena* is best considered a subspecies for the present. For a good figure of the new subspecies, see the reference of Yano, 1932, in Icon. Ins. Japan, Hokuryukan, p. 328; localities cited are incorrect except for Kyushu and possibly southern Honshu; as *C. herculeanus* var. *obscuripes*.

Worker (Holotype and paratypes): With the characters of typical *obscuripes*, and varying similarly in depth of the red of alitrunk and gastric base. The only difference noted is that *hemichlaena* has the entire prothorax black, whereas in the typical form, the prothorax is concolorous with the rest of the alitrunk or only slightly darker. Color best seen in alcohol.

Holotype [Entomological Laboratory, Kyushu University] 13. ix. 1939, Hikosan, Prov. Buzen, Kyushû, K. Yasumatsu leg.

124 *Paratypes:* [Museum of Comparative Zoology; U. S. National Museum; Entomological Laboratory, Kyushu University] see under distribution below.

Distribution: Kyushu and extreme southwestern Honshu, where it almost completely replaces *obscuripes obscuripes*. No intergrades between the two subspecies have yet been found, but they should occur in the junction zone in southern Honshu.

Localities: Honshu–Tsudamura, Prov. Aki; Gokurakujiyama, Prov. Aki; Taishakukyo, Prov. Aki. Kyushu–Sobosan, Prov. Bungo; Ichibusayama, Prov. Higo; Handa-plateau, Prov. Bungo; Kirishimayama, Prov. Osumi; Taradake, Prov. Hizen; Kuboteyama, Prov. Buzen; Inugatake, Prov. Buzen; Hikosan, Prov. Buzen; Inunakiyama, Prov. Chikuzen; Wakasugiyama, Prov. Chikuzen; Homansan, Prov. Chikuzen; Yakushima Island.

The nests are normally built in logs and stumps of conifers, especially pine and cryptomeria, in rather shady places. Sometimes the nests are made in the timbers of old buildings. Yasumatsu observed injury to timbers at the Hikosan Meteorological Observatory in 1937.

Camponotus yessensis Teranishi (Change of status)

Camponotus helcureanus [sic] *vagus* var. *yessensis* Teranishi, 1940, in Posthumous Section of Teranishi Memorial Volume, p. 72, ♀, original description, Hokkaido.

Camponotus herculeanus vagus var. *yessensis* Morishita, 1941, Mushi, **13**: 93; records from Honshu: Mt. Yakushidake, 400 M.; Mt. Hayachine, 550 M.; Kadoiri, Minoo District, 400 M.; Sugo, Yamashiro Prov., 360 M. Yasumatsu, 1941, Mushi, **13**: 96; record from Kyushu: Hikosan, 700–800 M.

This species is quite certainly a distinct species not very closely related to any other Japanese *Camponotus;* it is only superficially similar to *C. vagus* from southern Europe. The closest relationship is with *C. laevigatus* F. Smith from western North America, but *yessensis* is distinct from this species by pilosity and sculptural characters. Teranishi's species has very abundant fine, dense, erect pilosity over the entire dorsum of head and body and on antennal scapes and legs. True pubescence is nearly or quite absent from the gastric dorsum. The entire insect is black, nearly smooth and distinctly shining. Apparently it is widely distributed in the mountains of Japan at moderate eleva-

tions. The nests are built in rather dry logs and stumps, probably in situations similar to those in which *laevigatus* occurs in America.

Camponotus formosensis Wheeler (Change of status)

Camponotus maculatus taylori var. *formosae* Wheeler, 1909, Bull. Amer. Mus. Nat. Hist., **26**: 336, worker, original description, Formosa. Yano, 1910, Zool. Mag., Tokyo, **22**: 422, Formosa.

Camponotus herculeanus r. *punctatissimus* Forel (?), 1913, Arch. Natur., **79**: 200, worker, Formosa.

Camponotus barbatus albosparsa var. *formosae* Emery, 1929, Gen. Ins., **183**: 93, Formosa.

Camponotus punctatissimus formosensis Wheeler, 1929, Bol. Lab. Zool. gen. agr., Portici, **24**: 62, Taihoku. Sonan, 1939, Memorial Volume, 30 Years Anniversary of the Taiwan Gov. Mus., p. 212, Formosa.

Camponotus barbatus taylori var. *formosae* Teranishi, 1940, in Posthumous Section of Teranishi Memorial Volume, p. 60, Formosa.

A review of the types in the Museum of Comparative Zoology, Harvard University of this form and of additional specimens, all from Formosa, lead to doubts that *formosensis* can rightly be considered a race of Emery's enigmatic species *punctatissimus* (see under species inquirendae). There are no specimens referred to *punctatissimus* in any of the parts of Wheeler's original collection, and it is extremely doubtful that Wheeler knew any more about Emery's form than appeared in the *punctatissimus* description. If this is correct, we can see little correspondence between the Formosan specimens and the *punctatissimus* description. Emery mentions no unusual pilosity features, which he surely would have done had he been confronted with Wheeler's types.

Formosensis has very abundant coarse, yellowish-white erect pilosity on the dorsal surfaces of head and body, and the pubescence is remarkably long and dense, especially conspicuous and forming a closely appressed and slightly waved, anteriorly directed vestiture on the anterior dorsum of the alitrunk. The color is black and the sculpture coarse and opaque. This form appears to represent an extreme development of the tendencies shown by *japonicus*, but *formosensis* is so aberrant that we are forced to consider it a distinct species.

Forel had previously (reference not available) recorded a "*punctatissimus*" from Formosa, though we do not know the basis of his identification and consider it doubtful. Interesting enough,

Emery later (1925) raised *punctatissimus* to specific status, though he did not recharacterize it. The original description would lead one to believe that *punctatissimus* was very close, if not identical with *japonicus*. Until Emery's species has been better described and compared with close relatives, both it and *formosensis* must remain in some doubt. Meanwhile, we propose to consider *formosensis* a distinct species for practical reasons.

Species inquirendae

Camponotus punctatissimus Emery

Camponotus pennsylvanicus var. *punctatissimus* Emery, 1894, Ann. Mus. Stor. Nat. Genova, *34:* 477, ♀, Burma.

Camponotus punctatissimus Emery, 1925, Gen. In., Fasc. 183, p. 74, Burma, Tonkin, Formosa [?].

The discussion, see under *C. formosensis* above.

Camponotus cilicicus Emery, 1908, Deutsche Ent. Zeitschr., p. 186.

This species was briefly described by Emery, and we know nothing concerning it. Taurus was given as the original locality.

Camponotus vagus var. *kodorica* Forel, 1913, Ann. Soc. Ent. Belg., *57:* 145, Caucasus.

Camponotus herculeanus eudokiae Ruzsky, 1936, Trav. Inst. Sci. Biol. Tomsk, *2:* 90, Transbaikalgebiet.

We know this form only through the original reference, which both of us have not seen. If this species really belongs to the *herculeanus* complex, it is very probably just another addition to Ruzsky's already extensive synonymy in this group.

In the preparation of this paper, we owe a debt of gratitude to Dr. M. R. Smith of the U. S. National Museum for loaning the extensive collections of *Camponotus* made by D. C. Graham in West China and to Dr. J. C. Bequaert for facilitating examination of specimens in the Wheeler Collection. We wish to tender our grateful thanks to Prof. T. Esaki, of the Kyushu University, for the privilage of studying *Camponotus*-specimens in the collection of the University. We are also indebted, for the gift of specimens, to Messrs. K. Shibuya, R. Morimoto, S. Miyamoto, T. Miyake, S. Otsuka, Prof. M. Chujo, H. Okamoto, T. Ichiba, and Dr. T. Ishihara.

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WITCHES'-BROOM OF *PAULOWNIA TOMENTOSA* L.*

Yozan Tokushige†

Introduction

"Witches'-broom" of *Paulownia tomentosa* is not a disease of recent origin. T. Kawakami (1902) suggested that the disease was observed for the first time in a certain part of Kumamoto Prefecture in about 1880 and after that time it has gradually been spreading to various districts in Kyushu. Hazime Yoshii (1931 a) stated that the disease distributed in the southern parts of Japan including all Kyushu districts and southern parts of Korea, and was spreading to other districts. It was also found in North China in 1941. It seems that the disease spread to Kanto district in 1950. In these districts various protecting-measures have been taken without success to protect paulownia from this wide-spread and fatal disease.

The study on the disease had been undertaken by T. Kawakami (1902) in Kumamoto Prefecture. He discovered a kind of parasitic fungus on the petioles and the terminal buds of paulownia, isolated and cultured the fungus and studied its morphology, physiology and its parasitic nature. The results led him to the conclusion that it was the pathogenic fungus of the witches'-broom. The scientific name, *Gloeosporium Kawakamii* Miyabe was given to this parasitic fungus.

Hazime Yoshii attempted to ascertain the relationship of *Gloeosporium Kawakamii* with the witches'-broom of paulownia.

* Contribution from the Laboratory of Plant Pathology, Kyushu University.

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He studied on the fungus in respect to its morphology and nature in culture media, germinative physiology of the conidia, inoculation experiment, symptoms and anatomy of the infected paulownia. He came to the conclusion that *Gloeosporium Kawakamii* was not the pathogenic fungus of the witches'-broom of paulownia, but that the fungus was the causal organism of an anthracnose of paulownia (Yoshii, 1931 a, b, c; 1933 a).

Subsequently Hazime Yoshii (1933 b). assuming that the witches'-broom was a sort of virus disease, carried out graft inoculation. He succeeded in grafting and ascertained its infectious nature, but unfortunately the results were not observed for the successive years.

In 1946 the author started the experiment to confirm the graft-infectious nature of the witches'-broom of paulownia. In 1947 He succeeded in grafting four trees, —two of which showed the successful infection from diseased scions to healthy stocks.

In 1948 He tried to graft sixty trees and obtained the successful results of grafting infection. The results of the experiments

Fig. 1 Paulownia tree affected with "witches'-broom".

were reported at the Meeting of the Phytopathological Society of Japan (1948) and at the Meeting of the Japanese Forestry Society Kyushu Branch (1949).

Hiromu Yoshii (1950) of the College of Agriculture, Matsuyama, reported on the graft infectivity of the present disease, in which two of ten grafts showed the successful infection from diseased scion to healthy stocks. This report corroborates the authors' conclusion.

The present paper deals with the detailed results of the authors' experiments on graft infection of witches'-broom of paulownia.

Fig. 2. Witches'-broom symptom on the top of a diseased paulownia branch.

Symptoms

Typical symptoms of the disease are given in contrast with the normal trees.

(1) The growth habit

In the case of normal paulownia, a bud sprouts in spring and grows to a new stem or a new branch, which bears about ten

pairs of opposite leaves. The growth of stem ceases before the beginning of September. The axillary buds of the new stem or the branch do not sprout for this season, so that they usually have no lateral shoots for the current season (Fig. 3).

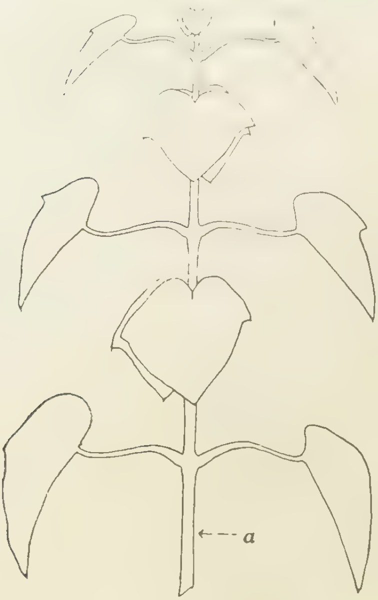

Fig. 3

Fig. 4

Fig. 3. The arrangement of leaves on normal paulownia. a: New stem.

Fig. 4. The arrangement of leaves and new shoots from resting buds on diseased paulownia. a: New stem or branch. b: Primary shoot. c: Secondary shoot.

In the case of the diseased paulownia, a bud sprouts in spring and grows to a new stem to a new branch which does not cease its growth until late in autumn. The primary axillary buds on the new stem or the new branch sprout and grow to the secondary shoots, then the secondary axillary buds to the third shoots and thus sprouting is repeated until late in autumn. The restlessness of the sprouting of the axillary buds and the growth of the shoots and branches without any restriction are the causes of the symptom of the witches'-broom.

(2) Branches

The branches and shoots of the diseased tree are slender and brittle and show an extreme negative geotropism. The color of diseased branches and shoots becomes yellowish green.

(3) Leaves and hairs

There are two sorts of leaf-forms in normal paulownia. One is the leaf-form seen in the young tree of one or two years old and the other is that seen in the elder tree. The former shows both large and small incisions on the margin of a leaf while the latter shows only large incisions (Figs. 5, 6). With the growth of the tree, the leaves which have only large incisions increase in number, and within 2 or 3 years the whole tree comes to have leaves with large incisions on the margin.

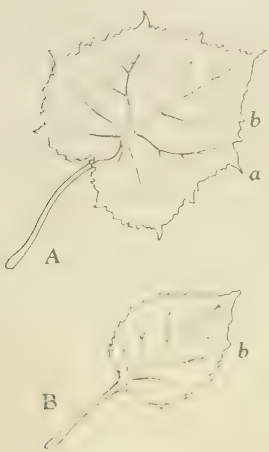

Fig. 5. The leaf-form on one or two years old normal paulownia.
A The leaf on uper parts of branch and stem.
B The leaf on lower parts of branch and stem.
a Large incisions.
b Small incisions.

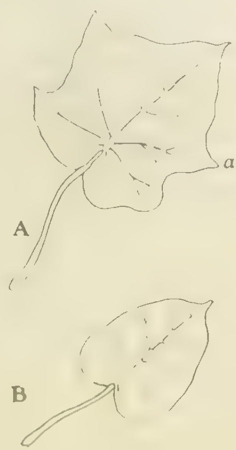

Fig. 6. The leaf-form on more than three years old normal paulownia.
A The leaf on uper parts of branch.
B The leaf on lower parts of branch.
a Large incisions.

In the diseased tree, however, the leaves with both large and small incisions appear even though it becomes more than two

years old (Fig. 7, left). Usually all the leaves on the diseased shoots are abnormally thin and narrow and are uneven on the surface. The color is faded to a yellowish here. Malformed leaves are often observed on the diseased shoots.

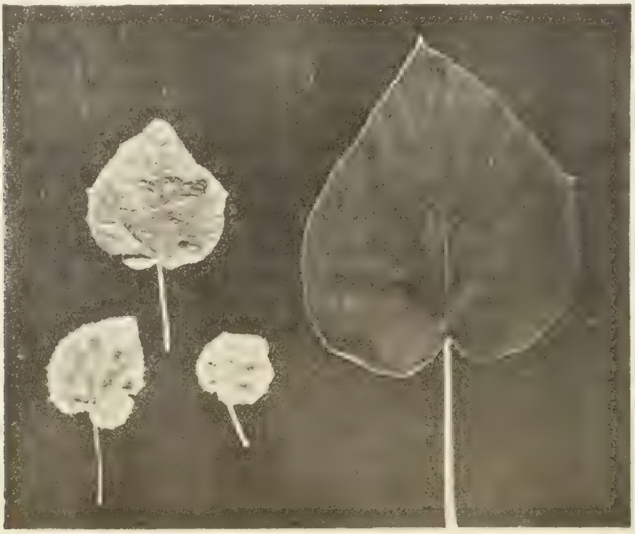

Fig. 7. A leaf from healthy plant (right) and three leaves from diseased plant (left).

There are four sorts of types of hairs on the under side of the leaves of paulownia (Fig. 8). The hairs which grow on the under side of the leaves on the healthy tree of more than two years old are mostly D type, and they grow very thickly. While most of the hairs which grow on the under side of the leaves on the diseased tree of more than two years old are those of A, B, or C type, and they grow sparsely.

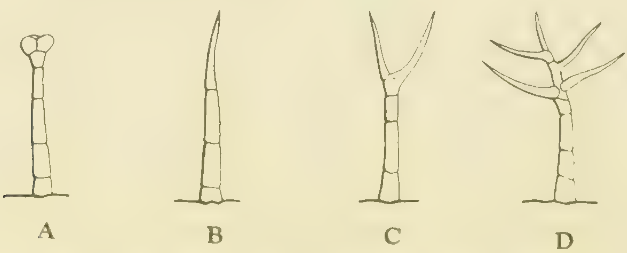

Fig. 8. The type of hairs on the under side of the leaf.

(4) Roots

The roots of the diseased trees are much retarded in growth. They are easy to decay; inner parts are discolored and become brownish. They have little regenerating power.

(5) Anatomical observation

No X-body has been found in any parts of the diseased tree. The cell menbrane is thinner and the nucleus shrinked and small.

A marked contrast is seen between the diseased and healthy trees in the differentiation of the woody cylinder. In the normal young tree, most of the large vessels are situated just out-side of pith and smaller vessels are scattered within the xylem elements (Fig. 9). On the contrary, in the diseased tree, many large vessels are also scattered within the xylem elements (Fig. 10).

Fig. 9. Cross section of healthy stem. × 50

The cell arrangement in the woody cylinder is irregular, especially around the vessels, and the rays are bent to and fro in the cross-section of the diseased stem.

(6) The formation of witches'-broom symptom

The diseased paulownia begin to show the characteristic symptoms of witches'-broom in May or June. The symptom is not restricted to the specific parts of a tree. It appears on the

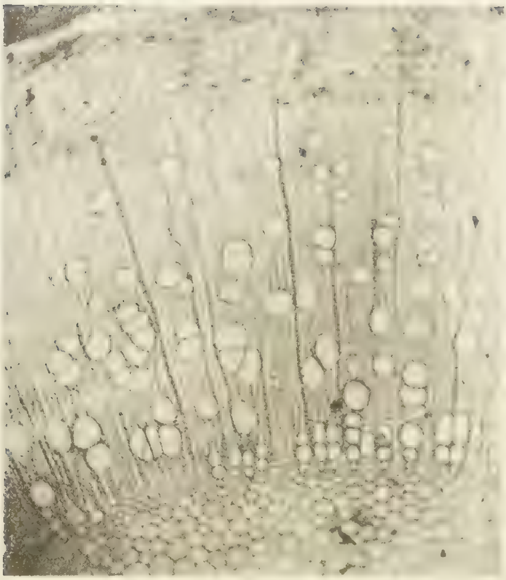

Fig. 10. Cross section of diseased stem. (×50)

lower or middle branch as well as on the top part of a tree and then spreads to the adjacent branches until the entire tree becomes affected. Many of the diseased leaves do not persist until late and begin to absciss in late summer or early in autumn. This abscission begins on the lower leaves of the diseased shoots and progresses upward as the season advances. The small leaves on the upper part of the diseased shoots, however, remain attached and the axillary buds continue to sprout late in autumn or even in winter.

In case the disease attacks the tree of one or two years old it may die within the current season or in the next season. In case the disease attacks the tree of more than three years old the growth power of the tree is strongly depressed. Even though they do not die within the current season, they will be dead within a few years.

Infection Experiments

Infection experiments were carried out by the means of grafting. Two methods of grafting were undertaken as follows: (1)

Grafting the diseased scion on the healthy stock (Fig. 11). (2) Grafting the healthy scion on the diseased stock (Fig. 12). If the symptoms of witches'-broom appear on the healthy stock in the former (1) case, or if the symptoms appear on the healthy scion in the latter (2) case, the witches'-broom of paulownia is an infectious disease. Experiments were carried out on the trees in pots which were placed in the green house.

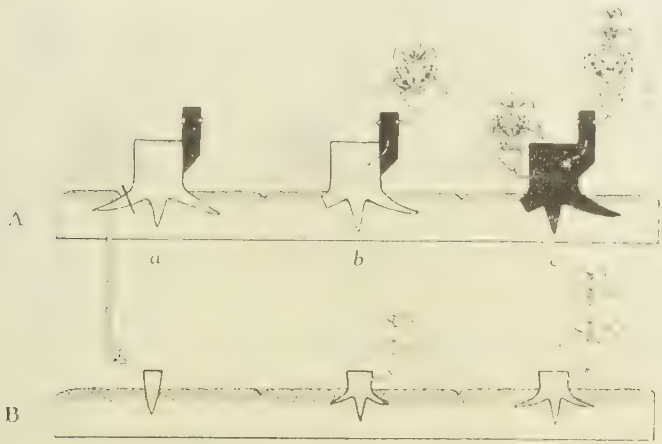

Fig. 11. Infection from the diseased scion to the healthy stock by grafting.

A Grafting the diseased scion on the healthy stock.
- a First state: Before budding.
- b Second state: Diseased symptom appeared on the grafted (diseased) scion.
- c Third state: Diseased symptom appeared both on the diseased scion and the healthy stock.

B Control from healthy root cutting.

1. Grafting the diseased *Paulownia tomentosa* scions on the healthy *Paulownia tomentosa* stocks.

In 1946, apparently healthy trees were selected from among the paulownia trees grown at the Fukuoka Forest Experimental Station. Seeds and root-stocks collected from these healthy trees were sown or planted in pots in the spring of 1947. In the next year, sixty two vigorous plants were selected from among the plants from seeds, and twenty-nine vigorous plants were selected from among the plants from root-shoots. On March 10, 1948,

thirty one healthy plants from seed were grafted with the same number of the scions, and the other thirty one plants were left without treatment for control. On the other hand twenty nine healthy plants from root-shoots were grafted with the same number of the diseased scions, while twenty-nine pieces of roots each of which was cut off from the root of grafted stock were planted in pots for control (Tables 1, 2, and Figs. 11—15).

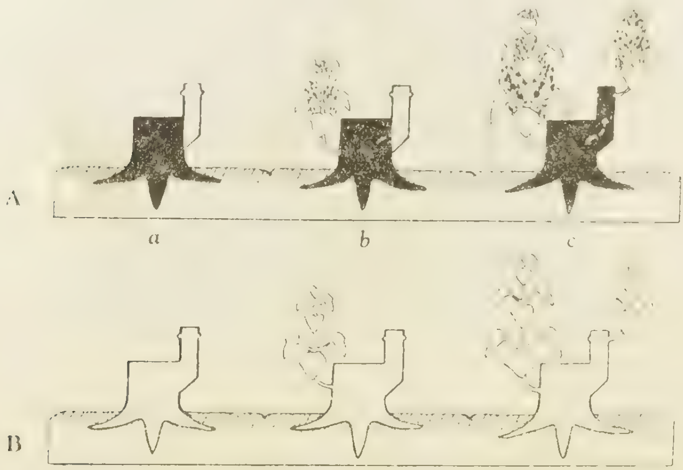

Fig. 12. Infection from the diseased stock to the healthy scion by grafting.

A Grafting the healthy scion on the diseased stock.

a First state: Before budding.

b Second state: Diseased symptom appeared on the diseased stock.

c Third state: Diseased symptom appeared both on the healthy scion and the diseased stock.

B Grafting the healthy scion on the healthy stock (check).

Table 1. Long observations on the graft-plants (grafted on March. 10. 1948): The diseased scions on the healthy stocks.

Plant no. \ Date of exam.		1948							1949						source of stocks
		30 IV	5 V	15 V	26 V	16 VI	26 VI	21 VII	3 VI	13 VI	23 VI	2 VII	12 VII	22 VII	
121	Gr	+	+	+	+	+	+	+							Se
	St	−	−					+							
128	Gr	+	+	+	+	+	+	+							
	St	−	−	−	+	+	+	+							

Plant no.	Date of exam.	1948							1949						source of stocks
		30/IV	5/V	15/V	26/V	16/VI	26/VI	21/VII	3/VI	13/VI	23/VI	2/VII	12/VII	22/VII	
138	Gr	−	−	+	+	+	+	+	+	+	+	+	+	+	Se
	St					−	−	+	+	+	+	+	+	+	
131	Gr	−	+	+	+	+	+	+							〃
	St					−	−	−							
130	Gr	−	−	+	+	+	+	+	+	+	+	+	+	+	〃
	St	−	−	−	−	−	−	−	+	+	+	+	+	[illegible]	
133	Gr	+	+	+	+	+	+	+							〃
	St	−	−	−	+	+	+	+							
115	Gr	−	+	+	+	+	+	+							〃
	St					+	+	+							
6	Gr	−	+	+	+	+	+	+							Ro
	St														
8	Gr	−	−	−	+	+	+	+							〃
	St	−	−	−	−	+	+	+							
4	Gr	+	+	−	−	−	−	−							〃
	St	−	−	−	−	−	−	−	−	−	−	−	−	−	
5	Gr	−	−	−	−	−	−	−	−	−	−	−	−	−	〃
	St			−	−	−	−	−							
3	Gr	−	+	+	+										〃
	St		−												
2	Gr	−	−	+	+	+	+	+	+	+	+	+	+	+	〃
	St				−	+	+	+	+	+	+	+	+	+	
1	Gr	−	+	+	+	+	+	+							〃
	St	−	−	−	−	−	−	−							
168	Gr	−	+	+	+	+	+								〃
	St														
188	U														〃
171	Gr	−	−	+	+	+	+	+	+	+	+	+	+	+	〃
	St														
156	Gr	−	−	−	−	+	+	+	+	+	+	+	+	+	〃
	St	−	−	−	−	+	+	+	+	+	+	+	+	+	
197	Gr	−	+	+	+	+	+	+	+	+	+	+	+	+	〃
	St					−	−	+	+	+	+	+	+	+	

Plant no.	Date of exam.	1948 30/IV	1948 5/V	1948 15/V	1948 26/V	1948 16/VI	1948 26/VI	1948 21/VII	1949 3/VI	1949 13/VI	1949 23/VI	1949 2/VII	1949 12/VII	1949 22/VII	Source of stocks
185	Gr	–	–	–	–	–	+	+							Ro
	St	–	–	–											
170	Gr	–	–	–	+	+	+	+	+	+	+	+	+	+	〃
	St	–	–	–											
30	Gr	–	–	–	–	–	–	+	–	–	+	+	+	+	〃
	St	–	–	–	–	–	–	+	–	–	+	+	+	+	
50	Gr	–	+	+	+	+	+	+	+	+	+	+	+	+	〃
	St		–	–	+	+	+	+	+	+	+	+	+	+	
57	Gr	–	–	–	+	+	+	+	+	+	+	+	+	+	〃
	St	–	–	–	–	–	–	+	–	+	+	+	+	+	
96	Gr	–	–	+	+	+	+	+	+	+	+	+	+	+	〃
	St	–	–	–	+	+	+	+	+	+	+	+	+	+	
26	Gr	–	–	–	–	–	–	–	+	+	+	+	+	+	〃
	St								+	+	+	+	+	+	
63	Gr														〃
	St														
19	Gr	–	–	–	+	+	+	+	+	+	+				〃
	St				–	+	+	+	+	+	+	+	+	+	
22	Gr	–	–	–	+	+	+	+							〃
	St	–	–	–	–	–	–								
49	Gr	–	–	+	+	+	+	+							〃
	St	–	–	–	–										
155	U														〃
89	Gr	–	–	–	–	+	+	+	+	+	+				〃
	St					+	+	+	+	+	+	+	+	+	
93	Gr	–	+	+	+	+	+	+							〃
	St					–	–	+							
101	Gr	–	+	+	+	+	+	+							〃
	St		–	–	+	–	–	–	–	–	–	–	–	–	
41	U														〃
31	U														〃

Plant no.	Date of exam.	1948 30 IV	5 V	15 V	26 V	16 VI	26 VI	21 VII	1949 3 VI	13 VI	23 VI	2 VII	12 VII	[illegible]	source of stocks
107	Gr	+	+	+	+	+	+	+	+	+	+				Se
	St		+	+	+	+	+	+		+	+	+	+		
105	Gr	+	+	+	+	+	+	+							″
	St	—	—	+	+	+	+	+							
109	Gr	+	+	+	+	+	+	+	+	+	+	+			″
	St	—	+	+	+	+	+	+	+	+	+	+			
199	Gr	+	+	+	+										″
	St			—											
114	Gr	+	+	+	+	+	+	+							″
	St	—	—	—	+	+	+	+							
201	Gr	+	+	+	+	+	+	+	+	+	+	+	+		″
	St	—	—	—	+	+	+	+	+	+	+	+	+		
180	Gr	+	+	+	+	+	+	+							″
	St	—	—												
205	Gr	+	+	+	+	+	+	+							″
	St	—	—	+	+	+	+	+							
75	Gr	+	+	+	+	+	+	+	+	+	+	+	+		″
	St					—	—	+	+	+	+	+	+		
113	Gr	+	+	+	+	+	+	+							″
	St														
110	Gr	+	+	+	+	+	+	+	+	+	+	+	+	+	″
	St	—	—	—	+	+	+	+	+	+	+	+	+		
73	Gr		—	—	—		—		—			—	—	—	″
	St														
156	Gr	—	—	—	—	—	—	—	—	—	—	—		—	″
	St														
16	Gr	—	—		—	—	—								″
	St								—	—	—		—		
182	Gr	—	—		—	—	—	—							″
	St			—	—	—	—	—	—	—	—	—		—	
125															″
122															

Plant no.	Date of exam.	1948 30 IV	5 V	15 V	26 V	16 VI	26 VI	21 VII	1949 3 VI	13 VI	23 VI	2 VII	12 VII	22 VII	source of stocks
17	Gr	−	+	+	+	+	+	+							Se
	St			−	+	+	+	+							
183	Gr	+	+	+	+	+	+	+							〃
	St	−	−	−	+	+	+	+							
140	Gr	+	+	+	+	+	+	+							〃
	St					−	−	+							
123	Gr	+	+	+	+	+	+								〃
	St														
132	Gr	−	−	−	+	−	−								〃
	St														
143	Gr	+	+	+	+	+	+	+	+	+	+	+	+	+	〃
	St	−	−	−	+	+	+	+	+	+	+	+	+	+	
136	Gr	+	+	+	+	+	+	+							〃
	St														

+…Symptom produced, −…Symptom not produced, Gr…Scion,
St…Stock, U…Unsuccessful grafting,
Ro…From root-shoot. Se…From seed.

Following results are obtained from Table 1.

(1) It took about fifty five days after grafting before the first symptom of witches'-broom appeared on the healthy stocks.

(2) The symptoms of witches'-broom appeared on the diseased scions at first and it appeared later on the healthy stocks.

(3) Only twenty seven of the total sixty graft-plants survived the winter.

The results show distinctly the progress of infection from diseased scions to healthy stocks.

The followings are obtained from Table 2.

(1) It is clearly observed that thirty of the sixty young-shoots were infected by grafting the diseased scions. Infection ratio is more than 90 per cent when the number of dead or unsuccessful graft-plants are taken out of consideration.

(2) Eighteen of the thirty one plants from seed were infected by the grafting the diseased scions. The infection ratio is almost 100 per cent when the number of dead or unsuccessful graft-plants are not considered.

(3) Twelve of the twenty-nine plants from root-shoots were infected by grafting the diseased scions. The infection ratio is about 80 per cent when the number of dead- or successful graft-plants are not considered.

(4) All the control plants from seed and root-shoots remained healthy except those root-shoots that died by careless management.

Above results clearly indicate the infectious nature of the disease from diseased scions to healthy stocks, though it has once been said that witches'-broom symptom of paulownia is the consequence of the repeated vegetative propagation.

Table 2. The results of grafting: the diseased scions on the healthy stocks.

Treatment	Seedlings	No. of plants used	No. of plants dead or unsuccessful grafting	No. of plants with symptom	No. of plants without symptom
Diseased scions grafted with healthy stocks	from seed	31	13	18	0
	from root shoots	29	14	12	3
	total	60	27	30	3
Not treated (check)	from seed	31	0	0	31
	from root-shoots	29	6	0	23
	total	60	6	0	54

2. Grafting the healthy scions on the diseased stocks.

Diseased materials were selected from among the paulownia trees that showed the witches'-broom symptom on the scions and stocks in the infection experiment in 1948. The healthy scions were grafted on five diseased plants from seed and on six diseased plants from roots on March 22, 1949. For the control, the healthy scions originated from above the same were grafted on five healthy plants from seed and on six healthy plants from root-shoots, which were selected from among the paulownia tree that were used for the checks in 1948 (Tables 3, 4, 5, and Fig. 12).

Fig. 13. Graft-plant (diseased scion on healthy stock.).
In the case of spring graft, the diseased scion on the healthy stock, the typical symptom appeared both on stock and scion in current summer.
D. Gr....Diseased scion, H. St...Stock which was healthy,
D. Sh...Diseased shoot from the stock.

Fig. 14. Control seedling.

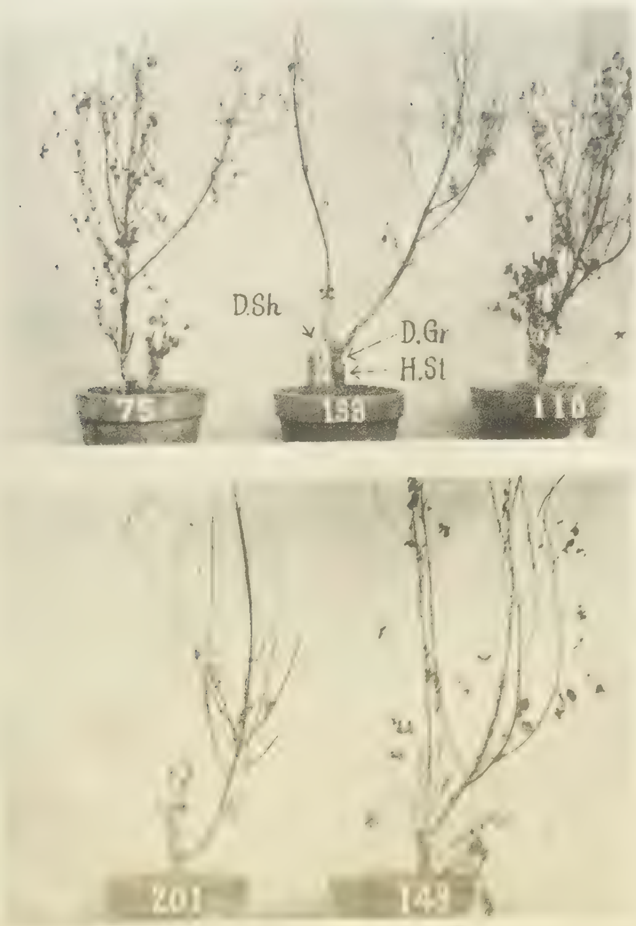

Fig. 15. Graft-plants (diseased scion on healthy stock).
In current winter.

Fig. 3. Long observations on the graft-plants (grafted on March 22, 1949): The healthy scions on the diseased stocks.

Plant no.	Date of exam.	1949							
		12/V	22/V	3/VI	13/VI	23/VI	2/VII	12/VII	22/VII
201	Gr	—	+	+	+	+	+	+	+
	St	+	+	+	+	+	+	+	+
75	Gr	—	—	+	+	+	+	+	+
	St	+	+	+	+	+	+	+	+
110	Gr	+	+	+	+	+	+	+	+
	St	+	+	+	+	+	+	+	+
143	Gr	—	—	+	+	+	+	+	+
	St	+	+	+	+	+	+	+	+
138	U								
6	U								
2	U								
156	U								
197	U								
30	Gr	—	—	—	—	—	—	—	—
	St	+	+	+	+	+	+	+	+
96	Gr	—	—	—	—	—	—	+	+
	St	+	+	+	+	+	+	+	+

+...Symptom produced, —...Symptom not produced, Gr...Scion,
St...Stock, U...Unsuccessful grafting,
Ro...From root-shoot, Se...From seed.

Following results are obtained from Tables 3 and 4.

(1) It took about fifty-one days after grafting before the symptom appears on the healthy scions.

(2) It was observed that the symptom appeared at first on the stocks and later on the scions.

The results show distinctly the progress of infection from diseased stocks to healthy scions.

Fig. 4. Long observations on the graft-plants (grafted on March 22, 1949): The healthy scions on the healthy stocks.

Date of exam. / Plant no.		1949							
		12/V	22/V	3/VI	13/VI	23/VI	2/VII	12/VII	22/VII
201′	Gr St	–	–	–	–	–	–	–	–
75′	Gr St	–	–	–	–	–	–	–	
110′	Gr St	–	–	–	–	–	–	–	–
143′	U								
138′	Gr St	–	–	–	–	–	–	–	–
6′	Gr St	–	–	–	–	–	–	–	–
2′	U								
156′	U								
197′	U								
30′	U								
96′	Gr St	–	–	–	–	–	–	–	–

Abbreviations are the same that of Table 3. The scions used in this experiment are of the same origin as that in Table 3.

Table 5. The results of grafting: The healthy scions on the diseased stocks.

Treatment	No. of plants used	No. of plants or dead unsuccesful grafting	No. of plants with symptom	No. of plants without symptom
Healthy scions grafted with diseased stocks	11	5	5	1
Healthy scions grafted with diseased stocks	11	5	0	6

Following results are obtained from Table 5.

(1) It is clear that five of the six healthy young-shoots were infected by grafting the diseased stocks, when five unsuccessful graftings were taken out of consideration.

(2) In the case of the control experiment, all six young shoots remained healthy through grafting on healthy stocks, when five unsuccessful graftings were taken out of consideration.

From the above results, it is obvious that the witches'-broom of paulownia is infectious from diseased stocks to healthy scions.

3. Grafting the diseased *Paulownia tomentosa* scions on the healthy *Paulownia Fortunei* stocks.

Ten root-shoots were taken in the spring of 1948 from the roots of the *P. Fortunei* which had been grown in the Faculty of Agriculture.

Four diseased scions of *P. tomentosa* were grafted on four healthy stocks of *P. Fortunei* on March 23, 1949. For the control, three healthy scions of *P. tomentosa* were grafted on three healthy stocks of *P. Fortunei* and three healthy root-shoots of *P. Fortunei* were left untreated (Tables 6, 7 and 8).

Table 6. Long observations on the graft-plants (grafted on Match 23, 1949): Diseased *P. tomentosa* on healthy *P. Fortunei*.

Plant no.	Date of exam.	1949 12/V	22/V	1/VI	11/VI	21/VI	1/VII	11/VII	21/VII
12	Gr	−	+	+	+	+	+	+	+
	St							−	+
11	Gr	+	+	+	+	+	+	+	+
	St		−	−	−	+	+	+	+
10	Gr	+	+	+	+	+	+	+	+
	St		−	−	−	+	+	+	+
18	U								

+ ...Symptom produced, − ...Symptom not produced, Gr...Scion, St...Stock, U ...Unsuccessful grafting.

Following results are obtained from Tables 6 and 7.

(1) It took more than ninety days after grafting before the witches'-broom symptom appeared on the healthy stocks of

P. Fortunei.

(2) The symptom appeared at first on the diseased scions of *P. tomentosa* and late on the stocks of *P. Fortunei* in the whole plants.

The data show distinctly the progress of witches'-broom infection from the diseased scions *P. tomentosa* to the healthy stocks *P. Fortunei.*

Table 7. Long observations on the graft-plants (grafted on March 23, 1949): Healthy *P. tomentosa* on healthy *P. Fortunei.*

Plant no. \ Date of exam.		1949							
		12/V	22/V	1/VI	11/VI	21/VI	1/VII	11/VII	21/VII
14	Gr	–	–	–	–	–	–	–	–
	St								
15	Gr	–	–	–	–	–	–	–	–
	St								
17	Gr	–	–	–	–	–	–	–	–
	St								

Abbreviations are the same as that of Table 6.

Table 8. The results of grafting: Diseased *P. tomentosa* scion on healthy *P. Fortunei.*

Treatment	No. of plants used	No. of plants unsuccessful grafting	No. of plants with symptom	No. of plants without symptom
P. Fortunei grafted with diseased scions.	4	1	3	0
P. Fortunei grafted with healthy scions (check I)	3	0	0	3
P. Fortunei not grafted (check II)	3	0	0	3

From Table 8 the followings are observed.

(1) All of the three healthy stocks of *P. Fortunei* were infected by grafting the diseased scions of *P. tomentosa*, when unsuccessful grafting was taken out of consideration.

(2) In the case of control graftings it is known that the symptoms did not appear on the healthy scions of *P. tomentosa* which were grafted on the healthy stocks of *P. Fortunei.*

Accordingly, it may be concluded that the witches'-broom of *P. tomentosa* is able to transmit to *P. Fortunei*, and that witches'-broom of *P. Fortunei* is not the results due to the stimulating action by the grafting with *P. tomentosa*.

4. Impossibility of the transmission of the disease through seed.

The seeds were collected from both healthy and diseased trees grown at the Fukuoka Forestry Experimental Station in 1949 and were sown in disinfected soil in pots in 1950. Twenty five pots were used to sow the seeds from the healthy *Paulownia tomentosa* and the same number of pots for the seeds from the diseased paulownia. About one hundred seeds were sown in each pot. No symptoms of the disease were observed on any of the seedlings obtained. Therefore, it seems that the witches'-broom disease of paulownia is not transmitted through seed so far as is shown in this experiment.

Discussion

It is shown that the symptoms of witches'-broom did not appear on any of the young-shoots of paulownia which had not been treated and on any of the graft-plants, healthy scions to healthy stocks. While, it is shown that the symptoms appeared on the healthy stocks as the result of grafting the diseased scions and that the symptom appeared on the healthy scions as the result of grafting the diseased stocks. Through these experimental data it is concluded that the witches'-broom of paulownia is an infectious disease. It is suggested from the following facts that it is a virus disease. (1) No visible parasitic organism has been found on the diseased materials. (2) The witches'-broom is a systemic disease. (3) Possibilities of infection were ascertained by grafting.

Paulownia Fortunei has bean considered as a possible resistant species, but it is found that it is also susceptible to the disease.

Summary

The witches'-broom of *Paulownia tomentosa* is a very severe disease and has long been known for its characteristic symptoms. The geographical distribution of the disease covers the southern parts of Japan and Korea. It is gradually spreading to other

districts at present. T. Kawakami stated in 1902 that *Gloeosporium Kawakamii* Miyabe was the pathogenic fungus of this disease. Hazime Yoshii proved experimentally in 1933 that the disease was not caused by *Gloeosporium Kawakamii.*

The experimental results of the present author are as follows.

(1) All eighteen plants from seed were infected by grafting the diseased scions.

(2) Twelve of fifteen plants from root-shoots were infected by grafting the diseased scions.

(3) Five of six plants from root-shoots were infected by grafting on diseased stocks.

(4) All of three *Paulownia Fortunei* were infected by grafting the diseased scions of *Paulownia tomentosa.*

From these experiments, it is concluded that the witches'-broom of paulownia is a virus disease, graft infectious, and that *Paulownia tomentosa* and *Paulownia Fortunei* are susceptible to the disease.

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AN IMPROVED METHOD OF CRYSTALLIZATION OF BEEF LIVER CATALASE

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On the way of purification of beef liver arginase, Kitagawa and author[1] obtained a crystalline protein and ascertained it to be identical with the crystalline catalase which had been first isolated by Sumner and Dounce[2] in 1937. In order to isolate the crystalline catalase, Sumner employed fractional precipitation by dioxane. An attempt to substitute acetone for dioxane was unsuccessful in those days. Dounce[3] reported in 1942 an acetone method which was almost identical in the principle with their original method.

Table 1. Crystalline catalase from various sources.

Source	Isolated by	Year	Kat. f.
Beef liver	Sumner, Dounce[2]	1937	26,000
Beef liver	Kitagawa, Shirakawa[1]	1941	32,000
Beef liver	Dounce[3]	1942	35,000
Beef erythrocyte	Laskowski, Sumner[4]	1941	48,000
Horse liver	Agner[5]	1938	62,000 (amorph.)
Horse liver	Dounce, Frampton[6]	1939	50,000 to 55,000
Horse liver	Anger[7]	1942	60,000
Horse erythrocyte	Anger[8]	1941	65,000 (amorph.)
Horse erythrocyte	Bonnichsen[9]	1947	65,000
Horse kidney	Bonnichsen[10]	1948	
Guinea pig liver	Bonnichsen[10]	1948	
Lamb liver	Dounce[3]	1942	
Human liver	Bonnichsen[10]	1948	
Human erythrocyte	Bonnichsen[10]	1948	50,000
Human erythrocyte	Herbert, Prinsent[11]	1948	
Bacteria (*Micrococcus lysodeikticus*)	Herbert, Prinsent[12]	1948	

Our method of crystallization of beef liver catalase, involving precipitation with acetone, heat treatment and salting out, was much improved there-after by further investigations.

At the stage of crystallization in the procedure, the precipitate obtained by salting out was dissolved in a small amount of water and soon after crystallization occurred. The fact in this case that the catalase crystallizes out from the same solution in which it once dissolved, is of much interest concerning the problem whether the crystalline catalase is of the same nature as in animal tissues or not. The change of the solubility of catalase at the stage of crystallization should be considered as to suggest an accidental change of chemical properties, and therefore the crystalline catalase might be of a different nature from the enzyme in the native state except the catalytic character.

The catalase capability of our preparations, in term of "Kat. f.", amounted to about 32,000, which was almost equal to that of Sumner's preparations, but much smaller than the value by Agner and Bonnichsen (Table 1). Attempts to increase "Kat. f." of preparations by means of recrystallization were in vain. Although many investigators ascribed the difference of catalase capability between horse and beef liver to the different hematin content, it was recently made clear that the crystalline catalase from beef liver contained almost the same amount of hematin iron as that from horse liver.[13] It is very important and interesting that catalase preparations have different capabilities according to their sources.

Catalase is generally supposed to be one of desmo-enzymes, which are bound to tissue cells. Bonnichsen[10] recognized recently that there was no essential difference immunologically between blood and liver catalases, and assumed that the source of liver catalase might be blood erythrocytes. But when the liver homogenate was placed under autolytic conditions, the amount of extractive catalase increased to a certain extent (about 40 per cent). This means that a considerable amount of catalase exists in the state of desmo-enzyme bound to tissue cells in the liver. The catalase capability of autolysate amounts to 350—400 units of "Kat. f.". On the assumption that the pure beef liver catalase has a capability of about 30,000, there must be about 1.2 grams of catalase in a liter of the extract. Nevertheless, in fact, only

0.1 gram of catalase was separated in the crystalline state by our method.

The solubility measurements are often useful for the confirmation of the purity of proteins and enzymes. Standing on the basis of the phase rule, the solubility test enables us to decide whether the solute is monodisperse in the solution or not. From the analysis of the solubility curve of the crystalline catalase, it was conclusively decided that the catalytic activity was essentially an attribute of the crystalline protein, and further the purity of the crystals as a protein was confirmed.

Experimental

I. Activity Measurement.

For the determination of reaction velocity the following system was employed,

0.02 N–H_2O_2	35 cc.	0°C.
M/30–Phosphate buffer (pH 6.8)......	10 cc.	
Enzyme solution (diluted)	5 cc.	

5 cc. enzyme solution (properly diluted) was added to the substrate mixture, from which 5 cc. was pipetted into 5 cc. of 2 N-sulfuric acid, and from this moment reaction intervals were measured. Similar aliquots of the reaction mixture were pipetted into 2 N-sulfuric acid at intervals, 2, 4, 6 and 10 minutes. The remaining quantity of hydrogen peroxide in each sample was determined by titration with 0.005 N permanganate. Catalase activity was expressed with monomolecular velocity constant at 0 time which was obtained graphycally by extrapolation of k-values[16].

$$k = \frac{1}{t_{\text{min.}}} \log_{10} \frac{a}{a-x}$$

Euler and Josephson[14] had been defined the catalase capability, "Kat. f.", as follows:

$$\text{Kat. f.} = \frac{k}{D_{\text{grams}}}\,.$$

Wherein D implies the weight of dry matter in 5 cc. of the properly diluted enzyme solution.

II. Crystallization of Beef Liver Catalase.

The improved method of crystallization and recrystallization of the beef liver catalase was summarized as follows:

Ground beef liver (1 kg.)

is treated with 1,000 cc. of distilled water and 5 cc. of toluene. The mixture is well agitated, allowed to stand over night in ice box, and then centrifuged (3,000 R.P.M., 40 min.). Fat membrane separated on the surface is removed.

Supernatant solution (about 950 cc.).

760 cc. of acetone is added slowly under cooling. After standing for 1 hour, the mixture is centrifuged (3,000 R.P.M., 30 min.).

Precipitate

is extracted with 300 cc. of water and insoluble matter is centrifuged off and 240 cc. of acetone is added to the solution under cooling. Centrifugation (3,000 R.P.M., 30 min.).

Precipitate

is dissolved in 150 cc. of water and the insoluble matter is centrifuged off. 20.7 g. of ammonium sulfate (recrystallized) is added slowly to the solution (about 0.25 saturation) and the precipitate formed is removed. 18.2 g. of ammonium sulfate is added again to the solution and after standing for 30 min. under cooling, the mixture is centrifuged (3,000 R.P.M., 20 min.).

Precipitate

is dissolved in 10 cc. of water and the insoluble matter is removed at once by centrifugation (4,000 R.P.M., 5 min.). The solution is allowed to stand in the ice box over night. Crystallization occurs ordinally in 30 min. and completes in one day. Centrifugation (4,500 R.P.M., 25 min.).

Crude crystals (needles; Kat., 1,000 to 1,500)

is washed 3 times with a small amount of water by centrifugation to remove ammonium sulfate and is suspended in 10 cc. of water. 0.1 N-NaOH is added drop by drop in the suspension until the thryxotropy* almost completely disappears (under pH 7.5†), and the insoluble amorphous proteins are centrifuged off.

Supernatant solution (about 10 cc.)

is titrated to pH 5.7 with saturated solution of KH_2PO_4 and is allowed to stand in the ice box. In 30 minutes crystallization occurs and completes in 12 hours. Centrifugation.

Crystalline catalase (plates; Kat. f., 10,000 to 15,000; yield, 0.1 g.)

The catalase capability can be lifted to about 30,000 by two or three times recrystallization. The crude crystals were usually needle shaped, but the recrystallized one was plates (Figs. 1 and 2). It appears that catalase crystallizes in plates from its concentrated solution near the isoelectric point when the salt concentration is comparatively low, and in needles when the con-

* The suspension of crystalline catalase shows a beautiful and silky appearance, which had been characterized by Sumner and Dounce[2] as "thryxotropy". By this characteristic one is able to ensure easily occurrence and solution of crystals.

† When stocked in ice box for weeks, crystals are often subjected to gradual denaturation and become insoluble at pH 7.5.

ditions are not optimum. When the needle crystalls were allowed to stand for a few weeks with its mother liquor, it yielded large and irregular shaped inactive crystals, which might be crystals of denatured catalase (Fig. 3).

Fig. 1. Crystalline Catalase (needles) ×250.

Fig. 2. Crystalline Catalase (plates) ×100.

Fig. 3. Denatured Crystalline Catalase ×400.

III. Autolysis of Beef Liver and Catalase Activity.

Attention was scarcely payed so far for autolysis in the purification of liver catalase. If catalase exists in the liver as a desmo-enzyme, it will be possible to increase the yield of crystalline catalase by a moderate autolysis of liver.

When ground beef liver was mixed with an equal portion of water and a small amount of toluene and then allowed to stand at 25°C., the amount of extractive catalase gradually increased together with the amount of soluble amino nitrogen, showing a maximum value (about 40 per cent increasement) at 20 hours' autolysis. At the maximum point the acidity of liver homogenate

reached to pH 5.3 and soluble amino nitrogen increased by about 20 per cent of total nitrogen (Table 2).

Table 2. The effect of autolysis of beef liver on catalase activity.

Intervals of autolysis	pH	Amino nitrogen		Activity† cmm. O_2/3 min.
		mg./cc.	%	
0	6.45	1.09	7.5	92
6 (hrs.)	6.40	1.24	8.5	98
12	6.02	1.75	12.0	119
18	5.53	2.30	15.7	127
24	5.20	3.06	21.0	128
48	5.00	4.99	34.0	96

* After van Slyke.

† Measured by manometric method.

Enzyme system: M/15-phosphate (pH 6.8) 3.00 cc.; 0.1 M-hydrogen peroxide 0.50 cc.; Enzyme solution 0.10 cc. } 30°C.

Unlike the case of the former method, the autolysis of liver homogenate yielded a clear reddish brown solution, which contained no colloidal suspensions. Hereby, the following manipulations of purification became considerably easy, but the yield of crystalline catalase decreased rather than by the usual method. To obtain crystalline catalase from the dialysate with better yield, the method was modified as follows:

Finely ground beef liver (1 kg.)

is mixed with 1,000 cc. of water and 5 cc. of toluene, and the mixture is allowed to stand for 20 hours at 25°C. Centrifugation.

Supernatant solution (about 950 cc.; Kat. f., 370)

is treated with 0.8 volume of acetone as before. Centrifugation.

Precipitate (Kat. f., 790)

is extracted with 250 cc. of water and the insoluble matter is centrifuged off. The solution is treated again with 0.8 volume of acetone. Centrifugation.

Precipitate

is extracted with 100 cc. of water. 2 cc. of 0.1 M-phosphate buffer (pH 7.2) and 2 g. of saliva are added to the solution. The mixture is allowed to stand for 10 hours at room temperature (18°C.). A calculated amount of ammonium sulfate is added to bring the solution to 0.25 saturation. Centrifugation.

Supernatant solution

is brought to 0.45 saturation by addition of ammonium sulfate. Centrifugation.

Precipitate

is extracted with 5 cc. of water and the insoluble matter is removed. In several minutes crystallization occurs.

Crude crystals (needles; Kat. f., 2,430; Yield, 0.1 g.)

Although, in this example, the yield of catalase was better than by the former method, it appears to require skill to obtain crystals by this method. It happened sometimes that catalase did not crystallize owing to obscure causes. The conditions of each step should be investigated still further.

IV. Solubility Test.

The measurement was performed according to the description of Northrop[5]. The thrice recrystallized catalase preparation (Kat. f., 32,100) was washed several times with water and further three times with 0.05 M-phosphate buffer (pH 6.5) and then was suspended in 15 cc. of the same buffer. This original suspension was successively diluted with the buffer as many as 4, 12, 36, 72, 144 and 288 times. 10 cc. of each suspension was pipetted into the centrifuging tube of 2 cm inside diameter and 25 cc. capacity, and agitated for 15 minutes with rotating stick (500 R.P.M.) to achieve the solubility equilibrium. After one hours' standing the suspensions were centrifuged (5,000 R.P.M., 20 min.) and then the catalase activity as well as the nitrogen content of the supernatant solutions were estimated by the usual method. All the operations were performed at room temperature. Considering the effect of temperature during centrifugation, four samples were centrifuged at the same time.

Table 3. Solubility measurements of the crystalline catalase (Kat. f., 32,100; pH 6.5; 18°C).

Dilution of the suspension	Activity k/cc.	Nitrogen mg/cc.
1	149	0.742
4	146	0.728
12	147	0.735
36	115	0.588
72	71	0.364
144	36	0.182
288	16	0.098

Results obtained were summarized in Table 3 and the typical solubility curve of crystals of maximum purity were illustrated in Fig. 4. The fact that the activity curve is almost parallel to the nitrogen curve should imply the identity of the carrier of these two characters. In Table 4 and Fig. 5 results of solubility measurements about three kinds of crystals with different purities were comparatively illustrated. These curves offer a further support to the conclusion above-mentioned.

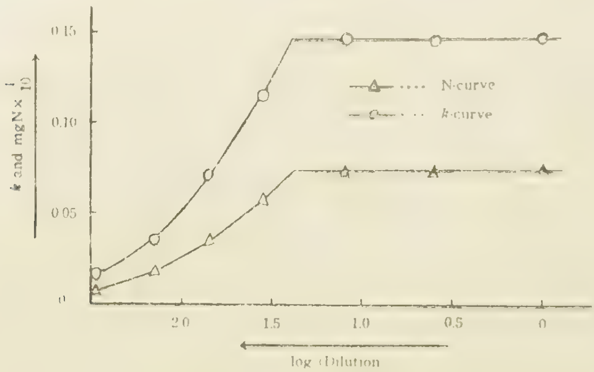

Fig. 4. The solubility curve of the crystalline catalase (18°C, pH 6.5).

Table 4. Solubility measurements of catálase preparations of various purities. (15°C, pH 6.5).

Dilution of the suspension	Preparation I (Kat. f., 1,230)		Preparation II (Kat. f., 24,000)		Preparation III (Kat. f., 30,500)	
	k/cc.	mg. N/cc.	k/cc.	mg. N/cc.	k/cc.	mg. N/cc.
1	40.6	3.94	142.2	—	130.6	—
4	37.2	3.41	136.4	—	128.0	0.714
12	30.9	2.90	128.6	—	128.6	—
36	15.1	1.93	117.8	—	92.2	0.539
72	7.7	1.03	57.4	—	52.2	—
144	4.0	0.52	30.2	—	25.4	0.146
288	—	—	—	—	14.6	—

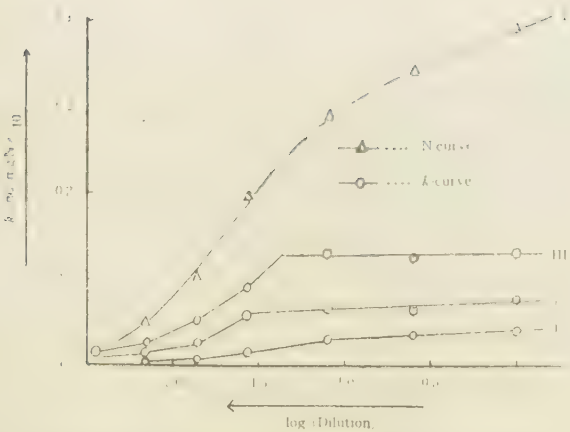

Fig. 5. Solubility curves of crystalline catalase preparations with different purities (15°C, pH 6.5).

Summary

(1) An improved method of crystallization and recrystallization of beef liver catalase was described.

(2) When the liver was autolysed at 25°C, the amount of extractive catalase increased by about 40 per cent during the first 20 hours, showing a maximum increasement.

(3) By some modifications of the technique, crystalline catalase was obtained from the autolysate with a better yield.

(4) By means of the solubility test, it was conclusively decided that the catalytic activity was essentially an attribute of the crystalline protein, and further the purity of the crystals as protein was confirmed.

The author is grateful to Professor Yasuyoshi Oshima for his continued help and advice.

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ON THE CHEMICAL CONSTITUENTS OF THE HEART WOOD OF *DISTYLIUM RACEMOSUM* S. ET Z.

(First Report)

TAMIO KONDO

INTRODUCTION

The discrimination of wood specimen is usually made by means of the difference of the constitutions of wood tissue, in other words, it is made from anatomical view points, but a few chemical methods, such as Mäule reaction,[2] fluorescence reaction[3] and flavone reaction, have been used as auxiliary means. Among these chemical methods, we prefer so called flavone reaction to others for the reason that this method has distinctive advantages such as that there are only a limited number of wood specimen which show positive reaction,[3] that its operation is simple and that its reaction can be observed with the naked eye.

The technical process of the method is as follows:[4]

The subject wood is sawed into fine dust and the dust is immersed in alcohol or methanol overnight from which alcoholic extract is separated. When a small volume of metallic magnesium or a few drops of mineralic acid is added to the extract with a few drops of mercury (or without it), it presents instantly reddish coloration. As the mineralic acid agent, we usually use sulphuric acid and hydrochloric acid, but sometimes organic acid such as acetic acid is found sufficient for the purpose.

Its tinct of coloration is usually reddish, but sometimes we observe only yellowish coloration. For example, in the case of alpinon which exists in "Semen Alpiniae" which does not possess free hydroxyl group in the side phenyl radical, it presents a yellowish coloration only.[5]

The chemical process of the flavone reaction is accounted for as follows: So called flavone substance in wood tissue is discharged into organic solvent. When it is reduced severely by hydrogen gas in the nascent state under the influence of mineralic acid, it is converted into so called anthocyan series which presents a distinct coloration

The substances which react positively on flavone reaction are limited to such series as flavone, flavonol (3-oxy-flavone, flavanone 2, 3, -dihydro-flavone), flavanonol (2-hydro-3-oxy flavone, and a few xanthone derivatives.[6] Therefore it was proposed that the term "magnesium-hydrochloric acid reaction" is more suitable than so called "flavone reaction" in the original sense of the word.

In the past, many studies on plant pigment have been mainly directed on its exterior tissues: such as flowers, leaves, roots and barks; and in addition, these studies have been originated from the interest of medicine and dyestuffs. Accordingly very little has been studied from the botanical standpoint for the classification of plant species, particularly of wood specimen.

However, recently an opinion was expressed that not only morphological characteristics of a plant but its constitutional features must be equally taken into consideration for the classification of plant species, so the idea of species should be considered from the standpoint of both morphological and physiological aspects.[8]

Such an idea was first expressed by Friedrich Boas in his work "Dynamische Botanik", where he termed it "das Souderstoff".[9]

On the other hand, Dr. Asahina and his co-workers have studied chemically and botanically moss materials in details, and utilized a special colouring reaction in the classification of its variety.[10]

In wood chemistry, the genus *Aesculus* is likely to contain aesculetin add all species of the genus *Fraxinus* to contain homologue fraxetin.[11]

Of course it is unreasonable to attempt the classification of plant-species basing only on their constitutional materials.

In the past, many of the chemical studies on wood have been carried towards the direction of the general chemical analysis.

It is worthy of closer attention that Erdmann and Nozoe recently discovered respectively thujaplicin (three α, β, γ-homologues) and hinokitiol, from woods of *Thuja plicata* Dovand and *Chamaecyparis taiwanensis* Masamune et Suzuki in their own way. Both of them belong to a type of keto-enolic cycloheptatrieneolone (tropolone series) which possess an antibacterial effect against wood decaying fungi.[12,13]

Literature of chemical studies on wood constituents is very scarce.[14,15,16]

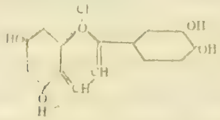
Now the term "flavone pigment" in this essay means the plant pigments which will be called "flavone" in a broad sense, containing flavonol, flavanone, flavanonol homologues.

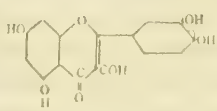
On the other hand a view on the plant physiological significance of flavone pigment by Shibata[7] has been generally admitted. To summarize his theory: these pigments, which dissolve in cell sap, absorb the ultraviolet rays of the sun light and accordingly protect protoplasma from a toxic action of these rays. In this sense the pigments are cell shelter against the ultraviolet rays. As the experimental foundations of this theory, it is emphasized as follows; first of all, these pigments are found universally in many higher plants, furthermore even in the same plant its content is more abundant in the exterior tissues than in the inner organs. The pigment-content in plant is known to increase according to the elevation of planted site in altitude. In addition, the recent spectroscopic studies have shown us the fact that these pigments strongly absorb the ultraviolet rays. However, recently the development of physical organic chemistry has revealed the facts that various natural organic substances show the absorption spectrum to the ultraviolet rays or infrared rays. Furthermore these pigments have been also detected in the underground organs, such as roots, hulls and even in heart wood of trees which are not directly affected by sun light.[17,18,19] Accordingly it is presumed as probable that these plant pigments would have much broader physiological bearing upon plant organs.

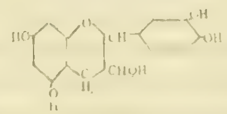
As to the physiological mechanism of the pigment formation in plant organ, now we are nearly ignorant on this subject as well as on the other materials. But from some simple consideration it has been assumed that flavone pigment is a forerunner of anthocyan pigment.[20]

When we turn our eyes to pharmacological effect of plant pigment, first of all we shall become aware of anthrachinone homologues which is recognized as a laxative and a urinating agent,[21,22] such as emodine and chrysophanic acid from *Rhizoma Rhei*, root of *Rheum palnatum* L. var *tanguticum* Maxim. Furthermore we shall notice a literature that flavone and its glucosides have also urinating effect.[23] Recently it was reported that pure ascorbic acid alone will have clinically no effect on a kind of blooding disease, but it will have an excellent effect on the patient when the dose is supplementarily with Vitamin P.[24] This V.P. (or citrin) was lately decided as a mixed crystal of a flavanone glucoside, hesperidin, and a free flavanone, eriodictyol.[25] A few years ago some interesting studies on rutin, which is a quercetin-3-rhamnoglucoside obtained from tobacco leaves, etc., were published in an American Technical Journal.[26] From the results of its clinical experiment it was recognized that this pigment has an excellent regulating action against the permeability of human blood vessels, namely to high blood-pressure disease and internal haemorrhage. And we know another physiological effect of rutin.[27] In America this pigment is now extracted from whole buckwheat glass and sold as rutin-tablet. Now in the field of medicine, the utilization of flavone pigment has been also closed up to our attention.

In the following scheme we can see the close chemical and physiological relationship between flavone pigments and anthocyane pigments or catechine series, which is one of the so called tannin substances.


cyanidin


quercetin


d, l,-catechin

Three substances mentioned above are structurally in oxidation and reduction type to each other. Really a flavone pigment, such as apigenine was reduced with sodium amalgam, acidified on addition of hydrochloric acid, and so the acquired precipitate was dissolved in alcohol, then added with ether, and the substance obtained in red needles was recognized as cyanidine.[28]

From the fact that both of these pigments are found in the same plant and also catechin is contained together with a corresponding flavonol,[29] there has been a proposal that it would strongly point to a physiological relationship among those substances,[30] but naturally these conclusions are doubtfull. However in a chemical laboratory reduction of flavonol into dl-catechin is attainable,[31] and the anthocyan series is converted into catechin through direct reduction.[32]

As given above the so-called flavone pigment contains four distinct types with regard to chemical structure.

flavon type   flavonol type   flavanon type   flavanonol type

All substances which belong to the four types are positive to the magnesium hydrochloric acid-reaction, giving bulky precipitates with lead acetate solution, and commonly breaking an oxygen bridge of γ-pyron through the introduction of caustic solution. The first three types had been found in plant organ since old times, but the last flavononol series, was lately discovered. Fustin from the heart wood of *Rhus succendanea* L. by T. Oyamada,[33] alpinon from the Semen Alpiniae, seed of *Alpinia japonica* Mig. by Y. Kimura,[34] ampeloptin from *Ampelopsis neliafolia* Kudo by T. Kubota,[35] and katuranin from *Cercidiphyllum japonicum* S. et Z. by H. Uota, T. Fukushima and T. Kondo[36] are all that belong to this type of substances which we are able to find in the literature hitherto published.

Dr. Nishida has maintained an opinion that on sulphite pulping the flavone pigment contained in the original wood will cause more or less pitch trouble, and recently a few studies were made from such a point of view.[38] In fact, *Larix Kaempferi* Sorg., *Cercidiphyllum japonica* S. et Z., *Distylium racemosum* S. et Z. are known as the most difficult woods on sulphite pulping, and contain a good amount of flavone pigment in each.

It should be noticed that such woods contain more or less flavone pigment which has such various significances as above mentioned.

Here the author attempted to identify chemically those positive substances to magnesium hydrochloric acid reaction in *Distylium*

racemosum S. et Z.

As chemical studies on *Distylium racemosum* S. et Z. we know only a few general analysis of wood[37, 38, 39] and ash-analysis,[40] for its ash has been utilized for a glazing mixture of "Arita ware".

In the test tube the author found that a sap wood contains scarcely such positive substances, so only heart wood was selected as material. This heart wood was sawed into powder, dried in the air. At first the author employed the usual lead acetate method as isolation process,[41] but his result was unsuccessful. The heart wood contained a great amount of tannin substance which possesses a similar constitution to flavone pigment and this catechol tannin would cause the isolation hinderance. Later he extracted pigments successfully from the saw dust with absolute ether in which tannin substance is insoluble. And two pigments were obtained from the ether extract, the one was recognized as quercetin that was first detected in the bark of *Quercus discolor* Ait.[42] and later isolated from many other plants. The other was here identified as 2-3-dihydroquercetin.

During and after the second world war we have been shut out from foreign literature, moreover even the domestic chemical publications are difficult to obtain. So the author will name provisionally the latter pigment as "Distylin" after its original plant name.

However recently the author obtained some American chemical literature, and among this found John Pew's study on "Douglas Fir Flavanone".[43] His so-called "Douglas Fir Flavanone" is identical with an optical isomer of the author's preparation, Distylin, according to his following experimental description. In addition, the author has come to be aware of shkimetin, which M. Takasaka and his co-worker isolated from a bark of *Illicium religiosum* S. et Z.[44] Shkimetin has an identical construction $C_{15}H_{12}O_7$ and assumed as quercetin-dihydro compound. But they described only one derivative, acetylshkimetin $C_{25}H_{22}O_{12}$ mp. 152.5° and no optical reference, nor further investigation in details was reported.

CHAPTER I

Isolation of Two Pigments

In plant chemistry we usually use the lead-acetate method as isolation process of plant constituents.[1] Especially has this method been favourably employed for the isolation of pigments, tannin substances, some kinds of glucosides and a few polyphenols. It is based on the following principle; these plant constituents, which show more or less acidic tendency in aqueous or alcoholic solution, react with lead acetate into its lead salts or an addition-product which is insoluble in water or organic solvents. These insoluble products are separated from some impurities, decomposed by dilute sulphuric acid or hydrogen sulfide into a free state.

Formerly the author extracted a new pigment from *Cercidiphyllum japonicum* S. et Z. with this method under the guidance of former Assistant Professor H. Uota.[7] In the preliminary experiment the author found that this isolation method was not successful on the above mentioned material. The heart wood of *Distylium racemosum* S. et Z. had a large quantity of tannin substance, which is likely to belong to a catechol group judging from a few coloring reactions. It may be easily imagined that its similar chemical construction to the pigments seems to cause the difficulty in isolating them from each other.

Distylium racemosum S. et Z. is a tall ever-green tree, belonging to the Hamamelidaceae family, which grows natively in the southern districts of Japan, Kyushu, Shikoku and Tsushima. Especially southern Kyushu has this abundant wood reserve. In April it blossoms and the fruiting season in October. This wood is very hard, since it is one of the heaviest woods in Japan. It is utilized as a material for house-building, utensils, furnitures, musical instruments, comb-manufacturing. Ash of the whole wood has been also used as a component of the glazing mixture of "Arita ware". Still more interesting to note is that in the mountainous region of Kyushu it is a popular thing to make a mimic sword from this wood and then season it in a paddy field for a few months. Through this treatment the sword surface becomes purplish black and the lusture is enhanced brilliantly. As a reason for the development of this coloration it may be properly con-

sidered that tannin substance, which is richly contained in the wood material, reacts with the ferric salt in a paddy field.

In consideration of the result of the preliminary experiment, the author extracted successfully with absolute ether, in which tannin substance is nearly insoluble. From the ether extract two crystals were obtained. Both crystals were positive to the magnesium hydrochloric acid reaction, one was a lemon-like yellow needle and the other a colourless crystal. The latter was provisionally named "Distylin" by the author.

In the literature we find a few researches on such pigments from the plant material. T. Oyamada isolated a yellow needle, fisetin $C_{15}H_{10}O_6$ and a colourless crystal, fustin $C_{15}H_{12}O_6$ from the heart wood of *Rhus succedanea* L.[33] Myricetin, yellow needle $C_{15}H_{10}O_8$ and ampeloptin, colourless crystal $C_{15}H_{12}O_8$, were isolated from *Ampelopsis meliscefolia* Kudo by H. Kubota through the usual lead-acetate method.[35] These experiments suggested that there is such similar relationship between both crystals obtained each by the author.

Experimental

The plant used in this experiment was contributed through the courtesy of the Tsuma Forestry Office, Kumamoto Forestry Bureau. The plant was used about one year after felling. Through cutting off sap wood only heart wood was collected. The heart wood was sawed into pieces and this saw dust dried in the air. The ground material was extracted in a continuous extractor with absolute ether for 7—10 days. Yellowish ether extract was evaporated on the water bath, and yielded a brown crystal mass containing some oily crust.

For removing the oily substance the crystal mass was extracted in Soxhlets with benzene until benzene extract becames colourless. The benzene insoluble mass was treated with hot water, divided into two fractions.

The one, insoluble part in hot water, afforded a brownish yellow mass. After several times of recrystallization from alcohol with charcoal, it gave fine yellow needles, m.p. 307—310°C under decomposition.

The other, in hot water soluble part, was allowed to cool to room temperature and gave fine leaflet crystals which sometimes

crystallized in needles. The colourless crystal was filtered, washed with cold water, and pressed on a porous plate. On recrystallization from hot water or dilute alcohol it became fine shiny leaflet crystal, m.p. 228—229°C under brownish decomposition. The yield of the yellow crystals was 0.7% to the weight of the original wood-powder and that of the colourless crystal was about 2.3%.

Chapter II

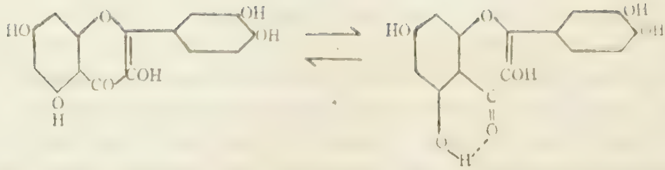
Identification of Quercetin

The hot water insoluble crystal is fine lemon-coloured needle and shows itself one of positive substances to the magnesium hydrochloric acid reaction in the wood material by its positive red coloration. It melts at the interval of 307—310°C with blackish decomposition, but the melting point is raised to about 315°C through acute heating.

From the results of its elemental analysis the molecular formula of the crystal is found in agreement with $C_{15}H_{10}O_7$. In addition some chemical behaviours suggest it to be quercetin, in the literature. To confirm this assumption the present author prepared a few derivatives as follows.

On the treatment with acetic anhydride and a drop of conc. sulphuric acid it gave a colourless needle, m.p. 197.5°C. This needle was identified with pentaacetyl-quercetin from the analytical figures and the mixed melting point determination with known one. Furthermore the author attempted the preparation of its methyl derivatives and when dealt with methyliodide and anhydrous potassium carbonate in acetone solution, a light yellow long needle was obtained. Through the same procedure it was decided to be tetramethyl-quercetin. The tetramethylquercetin has m.p. 155°C and is soluble in alkali. Its alcoholic solution shows positive reaction to ferric chloride solution. So the substance has more free phenolic hydroxyl groups.

It has been established in the literature that the free hydroxyl which is located in the para-position to the carbonyl radical is more resistently alkylized.[45] With regard to quercetin it will be properly proposed that it has the chelation structure which is to be expressed according to the following scheme.[46]



Such chelate ring may be more stable owing to hexacyclic formation, so it becomes more difficult to substitute the C_5-hydrogen atom by the alkyl group. The author used dimethyl sulphate as an alkylating agent.

In the literature γ-pyron ring in benzopyron molecule can be easily opened by alkali reagents.[47] So during the alkylating operation an excess of alkali should be strictly avoided. It was first successful when the same operation with dimethylsulphate and soda had been repeated three times under close observation.

The reaction product is a colourless prism, m.p. 148°C, insoluble in alkali, gives no coloration with ferric chloride solution and also sensitively melts at 112°C when recrystallized from dilute methyl alcohol. With the elemental figures it is recognized as pentamethyl-quercetin.[48] In the past, quercetin and its glycosides have been detected in many plants, but aside from the study on *Larix Kaempferi* Sorg.[49] we could not find any study of quercetin from wood materials.

Experimental

The lemon-coloured crystal is easily soluble in acetone, ether, hot alcohol, and pyridine, and is nearly insoluble in water, benzene and chloroform. Its alkali solution presents yellow coloration and gradually becomes brownish on standing. The alcoholic solution gives blackish green coloration with ferric chloride and also gives deep red coloration when reduced with metallic magnesium and conc. hydrochloric acid, with lead acetate solution it yields bulky red precipitate and strongly reduces Fehling's solution.

Anal. Sample was dried at the boiling point of toluene in Vacuum.

	Subst.	CO_2	H_2O	C %	H %
Found	16.7 mg	36.60 mg	5.50 mg	59.81	3.65
Calc. for $C_{15}H_{10}O_7$				59.60	3.31

Acetyl-derivative ;

The pigment was suspended in acetic anhydride and then was added to it a drop of sulphuric acid, it was dissolved into a reddish light yellow solution. Under stirring the reaction product was poured in to a great amount of cold water. With decomposition of acetic anhydride it changed to white mass, which after filteration was washed with water until no odour of acetic acid remained. On recrystallization from 95% alcohol it afforded fine colourless needles, m.p. 197.5°C. It showed no depression on the mixed melting point determination with known pentaacetylquercetin. The crystal was not soluble in alkali and gave no coloration with ferric chloride.

Anal. Sample was treated as above described.

	Subst.	CO_2	H_2O	C %	H %
Found	16.8 mg	36.0 mg	6.0 mg	58.48	3.96
Calc. for $C_{15}H_5O_7(COCH_3)_5$				58.59	3.96

Methyl derivatives:

1) Methylation with methyliodide and K_2CO_3

A little excess of methyliodide and anhydrous postassium carbonate was added to acetone solution of the pigment, refluxed on water bath for a few hours and filtered from the potassium salt. The acetone solution was evaporated to dryness and so the obtained crystal mass was recrystallized from methanol with active carbon. The methyl derivative was a light yellowish long needle, m.p. 155°C, soluble in alkali and gave a positive reaction to ferric chloride solution. No depression on the mixed melting point examination with know tetramethyl quercetin m.p. 156°C.

Anal. Sample (vacuum const.)

	Subst.	CO_2	H_2O	C %	H %
Found	18.9 mg	44.0 mg	8.9 mg	63.53	5.23
Calc. for $C_{15}H_6O_7(CH_3)_4$				63.68	5.02

2) Methylation with dimethylsulphate and NaOH

In methanolic solution of the pigment two times as much dimethyl sulphate was mixed, in which 10% soda solution was added drop by drop under severe stirring and careful precaution against its strong alkali reaction. After this procedure the product was still positive to the ferric chloride reaction. So this treatment was repeated three times on each product, and finally there was obtained a crystal indifferent to the ferric chloride reaction. Its

methanolic solution gave a light red tint on treating with metallic magnesium and conc. hydrochloric acid. When recrystallized from dilute methanolic solution, it melted at 112°C. Those qualities of the crystal were correspondent with one of pentamethyl-quercetin in the literature.[48]

Anal. Sample (vacuum const.)

	Subst.	CO_2	H_2O	C %	H %
Found	17.1 mg	40.1 mg	8.5 mg	64.00	5.52
Calc. for $C_{15}H_5O_7(CH_3)_5$				64.54	5.37

Chapter III

Physical and Chemical Properties of the Colourless Crystal

The colourless crystal obtained from the heart wood of *Distylium racemosum* S. et Z. formed itself into needles or plates depending on the conditions of recrystallization from water. However, when only needles were collected and recrystallized by the use of hot water they were occasionally obtained in plates. Kubota observed such dimorphism in the pigment of the homologous series, ampeloptin.[35] Generally there has been observed polymorphism on many inorganic compounds, but rarely on few organic substances, such as cumarin[50] and barbital.[51] Among natural organic compounds gallic acid,[52] chrysophanic acid[53] and morphine[54] were reported.

On heating in capillary tube it develops a light coloration at about 200°C, and becomes gradually deeper in tint and melts at 228—229°C under decomposition. As to the decomposition point the author observed some ranges depended on the rate of heating. It is raised to about 400°C by acute heating.

To confirm the purity of the crystal, acetylderivative, m.p. 153°C was prepared by the method which will be described in the next chapter and hydrolyzed with alcoholic sulphuric acid. The product was recrystallized from water in the same crystalline form as the original substance and melted at 228—220°C. However saponification with alcoholic kali was unsuccessful, because pyronring was broken by alkali reagents. So the crystal, m.p. 228—229°C is properly determined as a pure one for the following experiments. In the introduction the author named this crystal distylin.

It is soluble in hot water, methanol, alcohol, acetone and pyridine, insoluble in benzene, toluene, chloroform and cold water. It dissolves in bicarbonate solution, but no bubbling of carbon dioxide is seen. Its alkali solution is light yellow at first, and gradually becomes reddish violet. In conc. sulphuric acid it dissolves fresh yellow, but becomes colourless when a large quantity of water is added to it.

So the crystal develops a faint halochromy which may be considered as the characteristic colour reaction of unsaturated ketones. It gives the same colour reaction on addition of conc. sulphuric acid to the glacial acetic acid solution of the colourless pigment, but does not precipitate its oxoniumsalt. The aqueous solution gives a green coloration with ferric chloride solution, which becomes gradually blackish green. This green solution turns instantly into reddish violet when a drop of soda solution is added. Through this discoloration the crystalline substance suggested as a orthodiphenyl compound.[55] In the alcoholic solution it raises a blackish violet coloration with the ferric chloride solution and a light reddish with the metallic magnesium and conc. hydrochloric acid. But this coloration is also developed with granulated zinc and conc. hydrochloric acid, which has been lately reported as the characteristic of 3, 4-dihydroflavonol compounds.[13] Lead acetate solution gives voluminous dirty yellow precipitates. It reduces ammoniacal silver nitrate solution and Fehling's solution. The specific rotatory power was examined in acetone solution and alcoholic solution, but it was inactive in both solutions.

Through the above qualitative reactions the product was suggested as a substance of flavone series. Generally flavone pigments in plants were detected in forms of a free state or of glucoside. So the present author attempted to hydrolyse with mineral acid. The long action of dilute sulphuric acid to the product converted it in a yellowish crystal which was decided to be quercetin through the mixed melting point determination of its acetate with known pentaacetyl quercetin. The filtrate strongly reduces Fehling's solution after neutralization with potash. However the mother liquor was repeatedly extracted with ether and all reducing materials were found only in the ether extract. The ether extract was dried with Glauber's salt, evaporated to dryness

and the residue was divided into two fractions. The one was the unchanged original substance and the other was quercetin. So the reducing power of the mother liquor should be originated from two compounds, namely quercetin and colourless pigment. The mother liquor after ether extraction was neutralised with barium carbonate and evaporated to dryness. The residue was examined on organic compounds but no organic substance was recognized.

T. Oyamada got the corresponding dihydrocompound, fisetin, through long action of dilute mineralic acid to fustin under reduced pressure and normal pressure. So the author's colourless crystal is not glucoside but a substance which has a close structural resemblance to quercetin. The data of measurements of the molecular weight, water of crystallization and elementary analysis are in strict agreement with chemical formulation $C_{15}H_{12}O_7 \cdot 1\frac{1}{2}H_2O$.

Experimental

Measurement of the molecular weight:

The molecular weight of this substance was determined by Akiya's method,[56] which Professor Akiya modified dextrously the original Barger's method,[57] because the pigment dissolves in camphor under yellowish decomposition. As a solvent the author selected acetone and as standard substance azobenzene (m.p. 68°C) was charged.

1) Preparation of two solutions.

   a) the object solution: 0.304 g of the product was dissolved in 5 cc of acetone (60.8 g in Liter)

   b) the control solution: the standard solution of 0.3 molarity was prepared, in which 0.546 g of azobenzene (molecular weight 182) was dissolved in 10 cc of acetone. This original solution was diluted as follows:

No.	Original solution	Acetone (cc.)	Molarity	Corresponding mol. weight $M.W. = \frac{60.8 \text{ in L}}{\text{Mol.}}$
1	/	/	0.30	203
2	1.0	0.20	0.25	243
3	1.0	0.50	0.20	304
4	1.0	0.76	0.17	358
5	1.0	1.14	0.14	434

? Measurement: author employed a sliding microscope of 100 magnifications with ocular micrometer and measured a tube length which was located in the middle portion at the water temperature 24°C.

No.	M.W. $\frac{\text{min.}}{\text{length}}$ (mm)	0	60	145	215	590	Difference
1	203	8.30	8.15	7.98	79.8	7.55	−0.75
2	243	17.10	17.00	16.93	16.90	16.35	−0.75
3	304	13.39	13.35	13.38	13.35	13.35	−0.04
4	358	13.65	13.70	13.80	13.90	14.10	+0.45
5	434	15.70	15.80	15.80	15.80	16.10	+0.40

So the molecular weight was demonstrated as being between No. 3 and No. 4. Provisionally the mathematical average would be calculated as follows;

$$\text{M.W.} = \frac{304+358}{2} = 331$$

calc. for $C_{15}H_{12}O_7 \cdot 1\frac{1}{2}H_2O$ 331

Measurement of the water of crystallization:

The substance was dried at the boiling point of toluene in high vacuum for five hours.

Subst	283.9 mg	Loss in weight	23.2 mg	8.14%
〃	137.5 〃	〃	11.5 〃	8.36%
		Calc. for $C_{15}H_{12}O_7 \cdot H_2O$		5.60 〃
		〃 $C_{15}H_{12}O_7 \cdot 1\frac{1}{2}H_2O$		8.30 〃
		〃 $C_{15}H_{12}O_7 \cdot 2H_2O$		11.10 〃

Anal. Sample (vacuum const.)

Subst.	CO_2	H_2O	C %	H %
20.9 mg	45.3	7.7	59.15	4.09
19.9 mg	43.0	7.3	58.97	4.04
	Calc. for $C_{15}H_{12}O_7$		59.21	3.94

Action of dilute sulphuric acid on colourless crystal.

2 g of the substance were added in 100 cc of 3% sulphuric acid and the mixture gradually became a reddish violet to light yellow when slightly boiled on wire gauze for several hours. After fourteen hours it yielded crystalline mass in lukewarm condition. The mass was filtered and weighed about 0.35 g. The mother liquid gave an unchanged original substance, 0.6 g, on cooling. The crystalline mass was lemon-like yellow needles, on

recrystallization from alcohol it gave fine yellow crystals, m.p. 307°C. The alcoholic solution was positive to the magnesium hydrochloric acid reaction and developed a greenish brown on ferric chloride solution. On usual acetylation with acetic anhydride and conc. sulphuric acid it yielded colourless needles, m.p. 196°C. The acetate was identified as quercetin pentaacetate through the mixed melting point determination with known one.

When the mother liquid of quercetin and of the unchanged substance was treated with steam distillation the author accured no acidic distillate. The liquid strongly reduced Fehling's solution, but the reducing substance was transferred into ether layers, on repeating extraction with ether, after saturation with common salt. The ether solution was treated with the usual method and divided into two fractions. From the acidic fraction it gave an unchanged substance and from the phenolic fraction it yielded a few querce tion. So the reducing activity of the mother liquid must be considered as being originated from one of quercetin and the colourless pigment, because the residual liquid of ether extraction gave no organic substance after neutralization with barium carbonate.

Chapter IV

Derivatives of Distylin

Distylin has evidently the molecular formula, $C_{15}H_{12}O_7$ and belongs to the so-called flavone pigment (the carbon number of its standard structure = 15), because it develops an apparent magnesium-hydrochloric acid reaction. With regard to the function of oxygen it is obvious that two of them are pyroming's, so the remaining five must be determined up on its function. Therefore the author attempted to prepare its alkyl- and acyl-derivatives. At first the acetyl derivative was prepared; distylin was suspended in acetic anhydride and a drop of conc. sulphuric acid was added to it, then the product was recrystallized from methanol. A colourless needle was obtained, m.p. 153°C. The Mg-HCl reaction was indistinct in its alcoholic solution, but clear in the methanolic solution. The methanolic solution was also inactive to the ferric chloride solution. It was insoluble in alkali. From the result of this elementary analysis it is in agreement

with pentaacetyl-distylin. The author acquired the same product through the treatment with pyridine and acetic anhydride.

Then the benzoyl derivative was prepared by the similar method with that of acetylation described above and its product was a colourless needle, m.p. 200°C. The solution, in which the benzoyl product was dissolved in large quantity of alcohol, was positive to the Mg-HCl reaction. When the benzoyl product was once more benzoylated with pyridine and benzoychloride, but only the original substance was obtained. From the figures of elementary analysis it was confirmed that it was identical with pentaacetyl distylin $C_{15}H_7O_7(COC_6H_5)_5$. Furthermore the author obtained tetrabenzoyl-distylin $C_{15}H_8O_7(COC_6H_5)_4$, when benzoylation did not satisfactorily result. This product is a colourless needle and does not develop coloration to ferric chloride in its alcoholic solution. It melted at 192°C. The same tetramethyl-distylin was prepared through the usual three methylating agents: methyliodide and potassium carbonate, dimethyl sulphate and alkali, and diazomethane. It is a colourless needle, m.p. 170°C, inactive to ferric chloride in the methanol solution, and develops light reddish colour to the Mg-HCl reaction. From both the elementary analysis and the measurement of the methoxyl groups it is in agreement with $C_{15}H_8O_7(CH_3)_4$. The tetramethyl-distylin has evidently one more hydroxyl group, because the tetramethylmonoacetyl-distylin, m.p. 186°C was obtained in prismatic crystals through the acetylation of the tetramethyl-distylin with pyridine and acetic anhydride.

In the methanol solution it was indifferent to ferric chloride and positive to the Mg-HCl reaction.

From the results, of the above described experiments, it is evident that distylin has five hydroxyl groups in the molecule, four of them belong to the phenolic group and the last to the alcohlic group.

On the acetylation with acetic anhydride and sodium acetate the reaction product was a purple reddish substance which could not be crystallized from any other solvents. On the same treatment the flavanone homolog is generally converted into crystalline chalkone derivative through the cleavage of pyrone linkage.[illegible] On the other hand, the similar experimental observations with one on distylin were found in the reports of the other natural flavononol

pigments; ampeloptin and katuranin.[7, 8)] So it was suggested that the same constitutional relationship may exist between distylin and flavanonol compounds. The author could not obtain the oxime derivative through the usual method of oxime-preparation. However it is evident in the literature that on the flavanone compound the carbonyl group in pyrone linkage is active to the usual carbonyl reagents and gives oxime or semicarbazone,[9)] but the flavanonol pigment is generally inactive to the carbonyl reagents under the usual condition.

So distylin can not be considered as one of the flavanone group from this point.

Experimental

Pentaacetyl-distylin:

1) Distylin (0.2 g) was dissolved in pyridine (10 c.c.) and mixed with acetic anhydride being cooled all the while with flowing water. After standing for two days, the solution took on a brownish red coloration. The solution was poured with gentle stirring into a large volume of ice water. On further rubbing, the precipitated gummy substance soon became a white solid matter. It was filtered and washed with a little methanol. The insoluble substance in methanol was recrystallized by acetone and then not methanol. The acetyl product was colourless small needles, m.p. 153°C. The methanolic solution gives light pink coloration to the Mg-HCl reaction but the alcoholic solution gives none. It does not develop any coloration to the ferric chloride solution.

Subst.	CO_2	H_2O	C %	H %
22.8 mg	48.9 mg	8.6 mg	58.53	4.19
	Calc. for $C_{15}H_7O_7(COCH_3)_5$		58.36	4.28

2) Distylin (0.1 g) was digested with acetic anhydride (5 c.c.), to which was added a drop of conc. sulphuric acid under cooling. Floating substances became instantly deep red and soon dissolved in a yellowish solution. Holding the mixture at room temperature for several hours, and pouring it into ice-water, a solidified mass was separated. It was washed with a large volume of water and purified by redissolving in methanol. It melted at 153°C and no depression on the mixed melting point determination

with the preceding known pentaacetyl-distylin, m.p. 153°C.

Hydro-lysis of pentaacetyl-distylin (Regeneration of distylin):

At first the author attempted to saponify the product with alcoholic kali, but it was unsuccessful. After that it was effectively taken up to hydrolysis with alcoholic acid as follows: pentaacetyl distylin (0.3 g) was added to 3% alcoholic sulphuric acid and boiled in the water bath. In process of time it became yellowish, after 3 hours taken down and cooled on the table. An equal volume of water was poured in it and the mixture was extracted with ether, the ether extract was dried with anhydrous sodium sulphate and evaporated to dryness. The residue was purified from hot water with charcoal. The product was obtained in the same two crystalline forms, long needles and plates, with that of original distylin. It melted at 23. °C under decomposition.

Pentabenzoyl-distylin:

0.5 g of distylin in 3 c.c. of pyridine was gradually mixed with 3 c.c. of benzoylchloride under proper cooling. After two days the mixture was poured in to a large volume of cold water, it gave an reddish oily mass on the bottom. It was washed several times with dilute sulphuric acid to eliminate pyridine and a moderate quantity of alcohol was added with strong stirring. An insoluble part in alcohol was purified from acetone-water and then in acetone-alcohol mixture. The yield was weighed 0.8 g. It was colourless needles, m.p. 200°C. It gave a light red coloration in the solution of a large quantity of alcohol, to the Mg-HCl reaction and was inactive to the ferric chloride solution.

Subst.	CO_2	H_2O	C %	H %
17.2 mg	45.7 mg	6.6 mg	72.51	4.27
	Calc. for $C_{15}H_7O_7(COC_6H_5)_5$		72.81	3.88

Measurement of the molecular weight was executed by the Rast's camphor method.

Subst.	Camphor		$\triangle t$
2.08 mg	17.01 mg		6.0
	Found	M.W:	823
	Calc. for $C_{15}H_7O_7(COC_6H_6)_6$		824

Another benzoylation by the same method produced only the original pentabenzoyl-distylin, m.p. 200°C.

Tetrabenzoyl-distylin:

With the insufficient benzoylation, the author obtained colourless needles, m.p. 192°C. It gave also no coloration to ferric chloride in the alcoholic solution. It was assumed to be tetrabenzoyl-distylin from its analytical figures.

Subst.	CO_2	H_2O	C %	H %
18.5 mg	48.3 mg	6.7 mg	71.25	4.02
	Calc. for $C_{15}H_8O_7(COC_6H_5)_4$		71.68	3.88

Tetramethyl-distylin:

1) Methylation with methyl iodide: Distylin (0.5 g) was dissolved in acetone (20 c.c.) and then methyliodide (1.5 c.c.) and potassium carbonate (2 g) was added to the solution. The mixture was boiled in a water bath, and it gradually became fresh yellow. After 7 hours 1 c.c. of methyliodide was supplemented. Further it was reacted for 5 hours, filtered after cooling and the filtrate gave an oily mass on evaporation of acetone and unchanged methyliodide. The yellowish mass was recrystallized from methanol with charcoal. It gave colourless needles, m.p. 170°C. The methanolic solution gave no coloration to ferric chloride and developed a light coloration to the Mg-HCl reaction.

Subst.	CO_2	H_2O	C %	H %
17.2 mg	39.9 mg	8.4 mg	63.31	5.42
	Calc. for $C_{15}H_8O_7(CH_3)_4$		63.33	5.55

Measurement of methoxyl group;

Subst.	AgI	OCH_3 %
10.4 mg	26.8 mg	34.31
	Calc. for $C_{15}H_8O_7(CH_3)_4$	34.40

2) Methylation with dimethylsulphate: Distylin (0.5 g) was mixed with dimethylsulphate (3 c.c.) and then in the course of two days at room temperature 30% potassium hydroxide was introduced very gradually drop by drop with stirring. During the addition of the reagent the solution was kept faintly alkaline throughout with strict attention. The product was filtered, but, inspite of evasion from strong alkaline reaction, it appeared a reddish resin-like mass. On several purifications from methanol it gave colourless needles, m.p. 170°C in very small yeild, which was determined as tetramethyl-distylin through the mixed melting point determination.

3) Methylation with diazomethane: Nitrosomethylurea was prepared from potassium cyanate and methylamine hydroch-

loride[6] and utilized as the following methylation ragent. 1.3 g of distylin was suspended in 20 c.c. of ether and, on addition of ether solution of diazomethane which generated from 3 g of nitrosomethylurea, it severely reacted under bubbling of nitrogen gas. To dissolve the still unchanged substance, it was supplemented with more ether solution of diazomethane, which was prepared from 3 g of nitrosomethylurea. After standing over night, with fixation of calcium-tube, the ether solution was evaporated and the residue was treated with a small quantity of methanol. The insoluble portion in methanol was recrystallized from hot methanol with addition of water. It yielded the same methyl derivative, m.p. 170°C. In the soluble part in methanol, the author found one more colourless needle, m.p. 128°C. This product developed a brownish coloration to ferric chloride and also was active to the Mg-HCl reaction. It is assumed to be trimethyl-distylin, but the author could not determine this point from the analytical figures, because it was only obtained in a very small quantity.

Tetramethyl monoacetyl-distylin :

Tetramethyl-distylin (0.13 g) was dissolved in warm pyridine (2 c.c.) and acetic anhydride (2 c.c.) was added to it. After standing for two days, it was poured into a large quantity of ice-water, and the separated crystalline mass was filtered. It was purified from methanol in short prismatic crystals. It melted at 180°C. The methanolic solution gave no coloration with ferric chloride and was positive to the Mg-HCl reaction.

Subst.	CO_2	H_2O	C %	H %
19.3 mg	43.9 mg	9.0 mg	62.07	5.18
	Calc. for $C_{15}H_7O_7(CH_3)_4(COOH_3)$		62.68	5.47

Action of the carbonyl reagent :

Distylin (0.1 g) in alcohol was mixed with an aqueous solution, in which hydroxylamine hydrochloride (0.1 g) and anhydrous sodium acetate (0.15 g) were dissolved in two drops of water, and boiled on water bath for five hours. The mixture was liberated from alcohol and poured in ice-water. The separated white crystalline mass gave an original distylin, m.p. 230°C, which does not contain nitrogen atom.

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ON THE CHEMICAL CONSTITUENTS OF THE HEART WOOD OF *DISTYLIUM RACEMOSUM* S. ET Z.

(Second Report)

TAMIO KONDO

CHAPTER V

Degradation Study of Distylin

The author gives distylin the molecular formula $C_{15}H_{12}O_7$ and its oxygen-function is shown as follows: two of them belong to those of the γ-pyron ring, in which the carbonyl group in the C_4-position has more similar behaviour to one of the flavanonol type than of the flavanone type in the chemical reactivity and the remaining five are of hydroxyl groups, and four of them are aromatic and the last one is aliphatic, because tetramethyl distylin can be prepared with diazomethane, deprived of the ferric chloride reaction and of the solubility in alkali reagents.

The positive Mg-HCl reaction of distylin suggests that the number of the carbon linkage of the pigment is 15, but it is not conclusive, for a few xanthone derivatives are also active to the Mg-HCl reaction. However the expected carbon number 15 may be properly supported from the following experimental results: with the action of dilute sulphuric acid, distylin gave quercetin in good yield and no sugar was found in the filtrate. With regard to the attachment-positions of the hydroxyl groups in the molecule it is considered that there is perhaps a close relationship with one of quercetin and the author treated distylin with degradative cleavage in order to clear off this point.

In general the action of alkali to the pigment is appreciated on the structural study of the flavone substances. Really each group of the pigments, flavone, flavonol, flavanone and flavanonol stands on each special behaviour against alkali reagents, so we

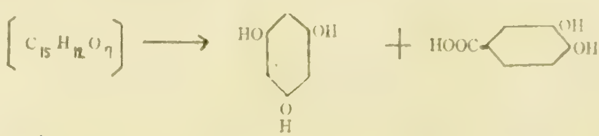
can obtain each characteristic reaction product from each peculiar degradative courses.[61]

On flavanone homologues the action of conc. alkali gives derivatives of phenol and cinnamic acid, but dilute alkali yields derivatives of ortho-oxy-acetophenone and benzaldehyde. On flavone homologues it gives 2-oxy-benzoyl-acetophenone as the first product, from which acid cleavage will finally come in chief with a small side reaction of ketone cleavage. So we obtain each one mol of phenol, acetic acid and benzoic acid with a little quantity of salicylic acid and acetophenone which originated from ketone-cleavage. On the action of strong alkali it raised up mainly acid-cleavage and also on weak alkali or alcoholic alkali it aroused ketone-cleavage.

On flavonol pigments the type of degradation differs from the two above described courses, it yields phenol and benzoic acid over the intermediate product of ortho oxy-acetophenone. Flavanonol pigments take a characteristic course of degradation as is described below.[33, 35]

In any cases it causes the cleavage of the γ-pyrone ring as the first step and by the action of strong alkali, in other words alkali fusion, we are able to obtain both phenol and phenol-carboxylic acid. Among them the phenol derivative is originated from the benzene ring of benzopyrone and phenol-carboxylic acid is also derived from the side-phenyl radical.

From the alkali-fusion of distylin the author obtained two substances in each crystalline form. The one was determined as protocatechuic acid and the other was identified as phloroglucin, respectively, through mized melting-point determination with known preparations. So it is reasonable to conclude that distylin has phloroglucin nucleus in benzopyronering and orthodioxy-phenol nucleus in side the phenyl radical.



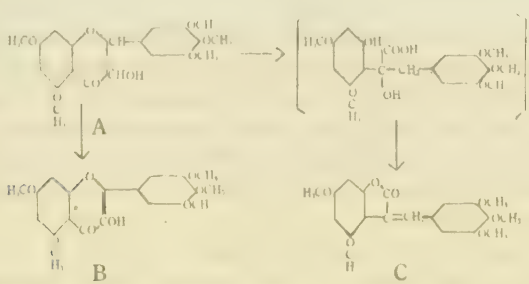
In the literature it is reported that quercetin gives the same two substances, phloroglucin and protocatechuric acid on the alkali-fusion. Here the author cleared off the constitutional relationship

and proved that distylin has the same structural components as that of quercetin.

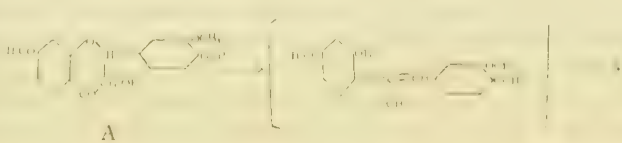
With the action of alcoholic potassium hydroxide, distylin gives protocatechuic acid and one more substance that crystallizes in colourless plates. The plates are insoluble in usual organic solvents and soluble in hot water. On ignition with a platin plate it leaves appreciable ash. The aqueous solution develops neutral reaction to the litmus paper, a green coloration to ferric chloride, and negative to the Mg-HCl reaction. From the results of the elementary analysis and the measurement of potassium it is in agreement with the molecular formula $C_{15}H_{13}O_{8}K.H_{2}O$. When it is heated in a capillary tube, it melts at about 210°C under decomposition.

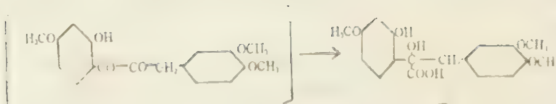
From the above described qualities the product can be recognized as the potassium salt of a phenol-carboxylic acid.

In the literature we find an interesting report that H. Kubota observed on ampeloptin from "shirocha" the following phenomena[35]: When pentamethyl-ampeloptin (A) was treated with 10% alcoholic potassium hydroxide for one hour, it gave anhydro-pentamethyl-ampeloptinlactone (C) with myricetin-5, 7, 3′, 4′, 5′-pentamethylether (B).



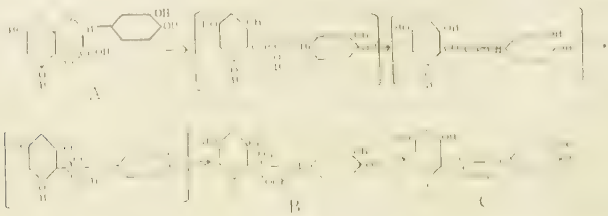
And also T. Oyamada treated trimethyl-fustin (A) with 10% alkali and obtained trimethyl-haseinic acid (B). So he illustrated this reaction in the following schema:[33]





B

It is generally recognized that such a cleavage-type is a characteristic for the flavanonol pigment.[61] With reference to these observations one may safely say that the formation of potassium salt of phenol-carboxylic acid from distylin took place as the product of α-diketone after the benzilic acid rearrangement. And so the author provisionally propose 3.4-dihydroquercetin (A) as the structural formula of distylin and according to this formula he illustrates this product in the following schema :



So the potassium salt (B) was dissolved in hot water, acidified with dilute sulphuric acid, on shaking out with ether, the ether extract yielded light brown short prismatic crystals, m.p. 228°C. The product dissolved in aqueous sodium bicarbonate solution under bubbling of carbon dioxide and the aqueous solution developed acidic reaction to the litmus paper and gave green coloration to ferric chloride.

And this green coloration changed into reddish violet on addition of a few drops of soda solution. It is inactive to the Mg-HCl reaction. From the figures of the elementary analysis it corresponds with the molecular formula $C_{15}H_{12}O_7$(C).

So the author attempted to obtain its lactone-derivative with dilute mineralic acid, but contrary to his expectation the original substance was only obtained. The reason of the unsuccessful ring-closure may be cleared off by the following explanation : in the cases of fustin and ampeloptin each methyl derivative was treated with alkali, so the two hydroxyl groups in 5.7-positions of

benzopyron nucleus were covered with methyl radicals, but on the other hand the author treated free distylin with alkali, so the two hydroxyl groups in 5,7-positions are mainly in the type of chinoide in acidic solvent.

If it is assumed that distylin will be formulated as 3, 5, 7, 3′, 4′-pentaoxy-flavone, its tetramethyl-derivative must be naturally 5, 7, 3′, 4′, -tetramethoxy-3-oxy-flavanone. About fifteen years ago Y. Kimura reported the 5, 7, 3′, 4′ tetramethoxy-3-oxy-flavanone, m.p. 176°C, which was synthesized through condensation of 2-oxy-4, 6, ω-trimethoxy-acetophenone and veratricaldehyde.[62] The interval between both melting points could not be diminished by the repeating recrystallization. Here we shall notice two asymmetric carbon atoms in the assumed 3, 5, 7, 3′, 4′-pentaoxy-flavanone molecule. So the existence of optical epimer is naturally considered, as in the case of crystalline catechine[63] that has close structural relationship with flavone pigment. Then the author treated natural tetramethyl-distylin, m.p. 170°, with 10% methanolic potassium hydroxide for a few minutes, and obtained one crystal, m.p. 176°C which has the same crystalline form, qualitative reactions with Kimura's 5, 7, 3′, 4′-tetramethoxy-3-oxy-flavanone.

Dr. Kubota reported the similar experiment on ampeloptin; on the treatment of pentamethyl-ampeloptin that derived from natural ampeloptin with 10% methanolic potassium hydroxide, he obtained a product which corresponded with synthetic pentamethyl-ampeloptin and he interpreted this phenomona by the opimerization.[65] According to Kubota's nomenclature this acquired product, naturally also synthesized tetramethyl-flavanone, should be named 5, 7, 3′, 4′-tetramethyl-epi-distylin.

Experimental

Alkali-fusion of distylin:

25 g of potassium hydroxide were weighed in the nickel basin, added to water (10 c.c.) and fused in the oil bath. In the fused alkali solution distylin (4.1 g) was thrown little by little with gentle stirring, and decomposed presenting a darkish red colour. The reaction was continued for 20 minutes at 190–200°C and for 10 minutes at 200—210°C. After cooling the mixture was added to a large volume of water, acidified with dilute sulphuric acid and extracted with ether. The darkish ether extract was

shaken with bicarbonate solution. The bicarbonate fraction was again extracted with ether, after acidification with dilute sulphuric acid. The ether extract was dried with Glauber's salt, and evaporated to dryness. The violet resinlike residue gave nearly colourless needles, m.p. 193°C. The aqueous solution of this product gave indigo coloration to ferric chloride and this coloration changed to red on the addition of the soda solution.

When lead acetate solution was added it gave a white precipitate which is soluble in acetic acid. It also reduced the ammonia-alkaline mercuric nitrate solution and strongly reduced Fehling's solution on heating. It was reliably identified as protocatechuic acid through the mixed melting point determination with known one that obtained from the alkali-fusion of quercetin.

The phenolic fraction, namely the ether extract free from the soda-soluble portion, gave a yellowish tar-like mass. Upon treatment with hot water the mass yielded a few plates of yellowish tar. The crystalline substance was smeared on a porous plate, and recrystallized by the use of hot water. When the product was dried at the boiling point of toluene it melted at 209°C. Its aqueous solution gave a bright violet colour to the ferric chloride solution, also a rose colour to the HCl-vanilline reaction, and a reddish violet to the fichten-span reaction. With the mixed melting-point determination with phloroglucin specimen that dried under the above described condition, no depression was observed (The mixed melting point: 213°C).

Action of methanolic potash:

Distylin (2 g) was dissolved in 40% methanolic potash (15 c.c.), the yellowish mixture became gradually brown on warming with the reflux condenser for one hour. After evaporation of the solvent it separated into colourless crystals with the lapse of time, which yielded about 1 g. It was recrystallized from hot water. The product was colourless plates that on heating changed to a light brown colour at about 180°C, became red at about 200°C and decomposed presenting a blackish brown colour at about 210°C. It was almost insoluble in the usual organic solvents and the aqueous solution was neutral to the litmus, and it developed green coloration to the ferric chloride solution. It gave voluminous yellowish white precipitate with lead acetate solution, reduces Fehling's solution and it was inactive to the Mg-HCl reaction. In

conc. sulphuric acid it dissolved into a brown solution, and this coloration though it became remarkably pale on addition of water, did not disappear. And the second addition of sulphuric acid gave again a deep brown coloration. After standing on the table for a long time its surface became a light reddish colour.

Sample: (air-dried)

Subst.	CO_2	H_2O	C %	H %
19.5 mg	33.7 mg	7.2 mg	47.25	4.12
Calc. for $C_{15}H_{13}O_8K.H_2O$			47.61	3.96

Measurement of potassium:

Subst.	K_2SO_4	K %
10.5 mg	2.4 mg	10.23
Cal. for $C_{15}H_{13}O_8K.H_2O$		10.31

Further, the mother liquor from the potassium salt, gave a little protocatechuic acid from its ether extraction.

The potassium salt in hot water was acidified with dilute sulphuric acid and extracted with ether. The ether solution was dried with Glauber's salt and yielded light brown crystals on evaporation of the solvent. The residue was purified from methanol, but remained only as oily substance. From using hot water it was successfully crystallized in light brown needles, m.p. 228°C. The aqueous solution was evidently acidic to the litmus. It dissolved in the bicarbonate solution under bubbling of carbon dioxide. And it gave no ash on ignition. In the aqueous solution ferric chloride gave a green coloration, which became a reddish violet colour on addition of soda. The author attempted to force the lactonering closure, but obtained only the unchanged original substance.

Subst.	CO_2	H_2O	C %	H %
14.7 mg	32.3 mg	6.07 mg	59.97	4.58
Calc. for $C_{15}H_{12}O_7$			59.21	3.94

Epimerisation of tetramethyl-distylin.

0.3 g of tetramethyl-distylin, m.p. 170°C was mixed with methanolic potash solution (3 c.c.) in which potassuim hydroxide (1 g) was dissolved in 50% methanol (10 c.c.), and the mixture was warmed on a water bath for 6 minutes. After cooling it was poured into a large quantity of ice water and acidified with dilute sulphuric acid. After standing overnight it separated into light reddish white precipitates. It was filtered and the product was

unfortunately separated from alcohol in milky emulsion. So the solvent was dried off and extracted with ether. The product from ether extract was kneaded on the porous plates with acetone. Through this procedure it was crystallized in a nearly colourless substance and purified two times from alcohol. It was light yellowish, sandy-formed crystals and melted at 176—177°C. The alcoholic solution was neutral to the litmus and gave no coloration to ferric chloride and to the Mg-HCl reaction. It was negative to both the phloroglucin-HCl and the vanilline-HCl reactions. In fuming nitric acid it dissolved into a bloody red colour. From the comparison of the melting point, the crystalline form and other colour-reactions it was identical with Kimura's synthetic 5, 7, 3′, 4′-tetramethoxy-3-oxy-flavanone. So according to the Kubota's precedenting nomenclature it should be properly named tetramethyl-epi-distylin.

Subst.	CO_2	H_2O	C %	H %
20.6 mg	48.0 mg	10.4 mg	63.58	5.65
Calc. for $C_{15}H_8O_7(CH_3)_4$			63.33	5.55

Chapter VI

The Structural Formula of Distylin

From the above described experimental results, the author proposed 3, 5, 7, 3′, 4′-pentaoxyflavanone as the structural formula of distylin. Now in comparing the chemical and physical properties of distylin with other flavanonol pigments, such as fustin, ampelopsin and Katuranin, the author will generally try to reexamine the reasonableness of his proposal.

1) Crystalline form and colourlessness of distylin. In general almost all of the natural flavone pigments are coloured and accordingly have been called "pigment" from this physical quality. Naturally some pigments are deep coloured and some light coloured. In the literature we find three groups of colourless substances which are called "pigment", the first is flavanone pigment, such as sakuranetin and butin, the second is a few glucoside of the pigment, such as acacetin, and the last is flavanonol pigment that was found in comparatively recent times. So the colourlessness of distylin suggest to us that distylin may be one of the above three groups. However in the inactivity of

the carbonyl group and the cleavage form with alkali reagent, distylin is considered neither glucoside nor flavanone. So distylin may be one of flavanonol homologues. Furthermore distylin has two crystalline forms, needles and plates under the crystalizing conditions. But it is not an unprecedented matter in flavanonol pigment, such as ampeloptin which is also in two crystalline forms. Now the crystalline forms and melting points of the known natural flavanonols are shown as follows:

Substance	crystalline form	m.p.
Fustin $C_{15}H_{12}O_6$[33]	colourless needles.	faintly brown at about 200°C, melts at 216−218°C under decomposition.
Alpinon $C_{17}H_{16}O_5$[34]	colourless long needles.	178°C
Ampeloptin $C_{15}H_{12}O_8$[35]	colourless needles or square plates.	245−246°C under decomposition.
Katuranin $C_{15}H_{12}O_6$[7]	colourless needles.	faintly colours at about 200°C, melts at 224−225°C under decomposition.
Distylin $C_{15}H_{12}O_7$	colourless needles or plates.	faintly colours at about 200°C, melts at 228−229°C under decomposition.

2) Coexistence of flavanonol with flavonol. It is notable that all known natural flavanonols have been always found together with those corresponding flavonols in the same plant tissue. In addition the content of flavanonol pigments is generally larger than flavonol's and its ratio is approximately 1:3. These simple experimental observations suggest to us an interesting physiological significance of the pigments in the plant metabolism. The following table shows the coexistence of both pigments and its round ratio.

Original plants	substance	yield		ratio	authors
Rhus succendanea L.	Fisetin $C_{15}H_{10}O_6$	of the raw subst.	20%	1	T. Oyamada
(in heart wood)	Fustin $C_{15}H_{12}O_6$	of the raw subst.	50%	2.5	
Amplelopsis neliafolia Kudo	Myricetin $C_{15}H_{10}O_8$	of the original material.	2.5%	1	H. Kubota
(in leaves)	Ampeloptin $C_{15}H_{12}O_8$	of the original material.	7.4%	3	
Alpinia japonica Miquel	Izalpinin $C_{16}H_{12}O_5$			1	Y. Kimura
(in seeds)	Alpinon $C_{17}H_{16}O_5$			4	M. Hoshi

Gericidiphyllum				
japonicum Š. et Z.	Kaempferol $C_{15}H_{10}O_6$ of the material.	0.03%	1	H. Uota
			:	T. Fukushima
(in wood)	Katuranin $C_{15}H_{12}O_6$ of the material.	0.10%	3	T. Kondo
Distylium racemosum				
S. et. Z.	Quercetin $C_{15}H_{10}O_7$ of the material.	0.7%	1	
			:	T. Kondo
(in heart wood)	Distylin $C_{15}H_{12}O_7$ of the material.	2.3%	3	

3) Distylin has an intermediate quality between flavonol and flavanone in chemical behaviour. On flavanone compound its carbonyl group of γ-pyrone is usually active to the carbonyl reagents and gives its oxime and semicarbazone. And with conc. sulphuric acid flavanonol yields an oxonium compound in the glacial acetic acid solution. Distylin gives no carbonyl derivative through the usual treatment, so from this result it may be considered rather as one of flavonol homologues. But it also gives no characteristic oxonium salt of flavonol pigment. So the chemical quality of distylin must be considered as being in the intermediate position between both pigments.

4) Distylin has the characteristics of flavanonol pigment. Through the action of dilute mineralic acid, or rarely of dilute alkali, flavanonol compound is converted into the flavonol. Accordingly it will be reasonable to assume that distylin is a corresponding dihydro-derivative of quercetin, because it gives quercetin with the action of mineralic acid. The second characteristic of flavanonol was observed on the acetylation with sodium acetate and acetic anhydride. In this treatment it gives an amorphous reddish substance that could not be obtained in crystalline form. Distylin gives the same reddish substance under the same condition of acetylation. However flavanone yields crystalline chalkone derivatives on the same acetylation.

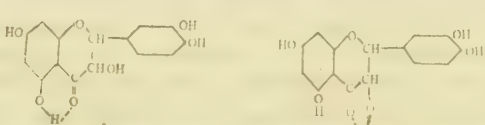
The cleavage-type of the pigment, which is developed by alkali reagent, is the most characteristic aspect of flavanonol homologues. It appears to be produced 1.2-diketone derivative as the intermediate product of this reaction, and we obtain one phenol-carboxylic acid as the final product of the secondary reaction of like-benzilic acid rearrangement.

Distylin gave one phenol-carboxylic acid that may be explained by the above mechanism.

Furthermore it is reported that 3-oxy-flavanone compound develops reddish coloration on the treatment with granulated zinc and hydrochloric acid,[1] while distylin is positive to this color reaction.

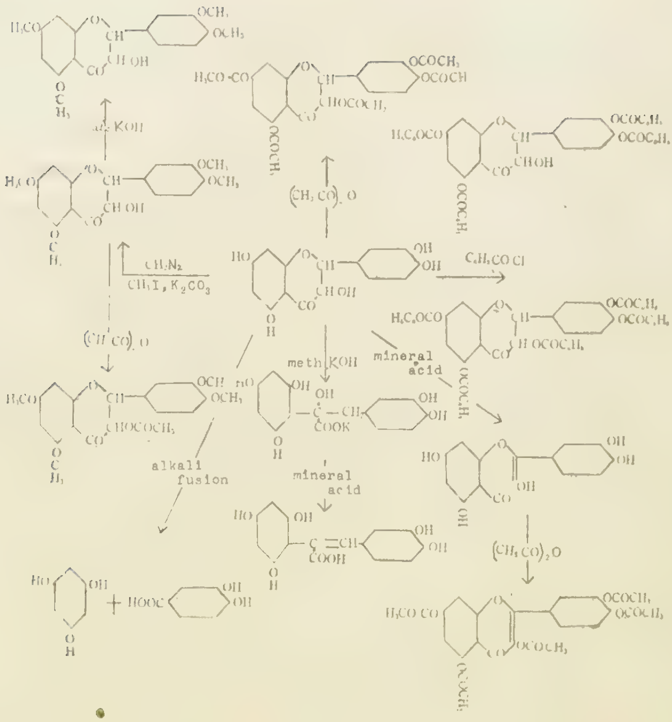
5) The hydroxyl group, in the 3-position of distylin, is an alcoholic one. Five hydroxyl groups of distylin are divided in two forms, to one of which four belong to the phenolic group and the other to the alcoholic group. And from the fact that tetramethyl derivative was barely obtained, when an excess of diazomethane was employed on the methylation, it is obvious that one of the four phenolic hydroxyls resisted to the methylation.

So this one group is perhaps in the 5-position of the benzopyron linkage, in other words in the ortho-position to the carbonyl radical. On the other hand the hydroxyl group in the 5-position of flavonol resists strongly the methylation and it is barely methylated by the treatment with dimethyl sulphate and potassium hydroxide. But in the literature we find no reports that the hydroxyl group of the 3-position resists its methylation. Accordingly the resistence of the 3-position-hydroxyl group to the methylation may be explained as follows: the hydroxyl group may be considered with high probability as one of dihydro-γ-pyron linkage, so flavanonol compound acquires increasingly hydroaron acity with the loss of aromaticity in its chemical activity. So beside hexacyclic linkage (A), the formation of pentacyclic linkage by the hydrogen bond (B) may be assumed, accordingly the methylation of 3 hydroxyl group becomes troublesome and simultaneously the resistance of the 5-hydroxyl group may appropriately diminish.



A B

6) On the ground of the assumption that distylin is 3, 5, 7, 3′, 4′-pentaoxy-flavanone, the above described experimental results are abstracted in the following scheme.

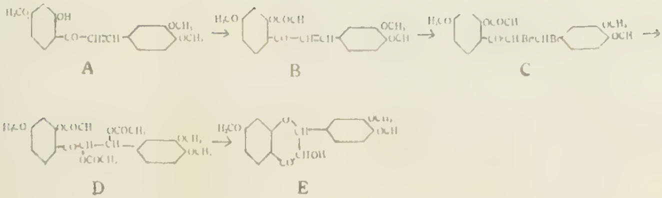


Chapter VII

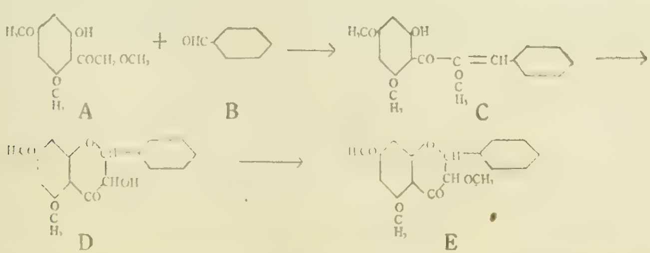
Synthesis of Distylin

There have been reported two different synthetic procedures of the flavanonol pigment. The one was executed by T. Oyamada as follows[38]: He choosed 2-oxy-4, 3′, 4′-trimethoxy-chalkone (A) as the first material for his synthesis. The chalkone (A) was acetylated with acetic anhydride and anhydrous sodium acetate, and converted into 2-acetoxy-4, 3′, 4′-trimethoxy-chalkone (B). Through the biomination of the product in the carbon bisulfide solution it gave 2-acetoxy-4, 3′, 4′-trimethoxy-chalkone dibromide (C). For the substitution of bromine with acetyl group the halide was treated with silver acetate in the acetic anhydride solution, and thus obtained 2, α, β-triacetoxy-4, 3′, 4′-trimethoxy-chalkone (D) was saponified and simultaneously closed in γ-pyron compound.

trimethyl fustin (E), by the action of dilute mineralic acid in the alcoholic solution.



In the literature we find one more method, which Y. Kimura performed on the synthesis of apoalpinon-dimethylether.[62] Namely he started from 2-oxy-4, 6-dimethoxy-ω-methoxy-acetophenone (A) and benzaldehyde (B). Through condensation of both compounds with the existence of alkali, 2-oxy-4. 6-dimethoxy-α-methoxy chalkone (C) was prepared. The chalkone was treated with the dilute solution of hydrochloric acid in alcohol for a long time and 3-oxy-5. 7-dimethoxy-flavanone (D) obtained which was finally methylated with dimethyl sulfate and alkali. This methyl derivative was recognized as apoalpinon-dimethylether (3, 5, 7-trimethoxy-flavanone) (E).



Independent of Kimura's study, H. Kubota synthesized 5, 7, 3′, 4′ 5′-pentamethyl-epi-ampeloptin with the same method.[45] And he found that on the ring-closure of chalkone with alcohol-mineralic acid demethylation of the 3-position did not occur under some experimental conditions.

The author proposed 3, 5, 7, 3′, 4′-pentaoxy flavanone as the structural formula of distylin and attempted to synthesize this compound by Kimura's method and set himself to this work. Namely he started from the cleavage of pentamethylquercetin

with alcoholic potash and obtained 2-oxy-4, 6-dimethoxy-ω-methoxy-acetophenone and 3.4-dimethoxy benzoic acid and from the latter he prepared 3.4-dimethoxy-benzoyl-chloride with the intention of synthesising 3.4-dimethoxy-benzaldehyde. However just at this time the author noticed the original report in which J. C. Pew has made from his studies on the pigment of Douglas Fir.[43] From the heart wood of this tree he obtained an optically active flavanonol, d-3, 5, 7, 3', 4'-pentaoxy flavanon and finally synthesized the corresponding racemic compound through direct reduction of quercetin. On various qualities of this racemic compound described in his report the pigment of Douglas Fir corresponded well to the one of distylin, so the author changed his synthetic project and synthesised his preparation with Pew's simple method. Accordingly the solution of quercetin in sodium carbonate was strongly reduced with sodium hydrosulfite and the product obtained was strictly identical with distylin in the physical and chemical properties. Furthermore it was also proved from the following experiment: the product was acetylated with acetic anhydride and pyridine, and acetyl derivative (m.p. [illegible]°C) was mixed with penta-acetyl distylin and no depression of the mixed melting point was observed. Accordingly it is doubtless that distylin is racemi-Douglas Fir-flavanone, namely dl-3, 5, 7, 3', 4'-pentaoxyflavanone.

Experimental

Reduction of quercetin with sodium hydrosulfite: Synthesis of distylin. Quercetin (2.0 g) was sufficiently mixed with sodium carbonate (17 g), added to water (200 c.c.) and the mixture was dissolved in the homogeneous solution under warming in water bath. Sodium hydrosulfite (40 g) was added to the homogeneous solution in each 2–3 grams, under bubbling of hydrogen sulfide it was warmed in water bath. After fifteen minutes the mixture was added to 250 c.c. of water and some dilute sulfuric acid until a violet brown coloration of hydrosulfurous acid remained for some time. On cooling it separated into some precipitates of unchanged quercetin, filtered off and the filtrate was extracted with ether. The ether solution gave a light brown mass on evaporation of the solvent. On standing in the $CaCl_2$ desiccator under diminished

pressure it yielded light yellow crystals. The yield was 0.32 g, equal to 16% of the weight of the quercetin used. The product was recrystallized several times from hot water and developed into the form of plates or needles under conditions of recrystallization. On heating it lightly coloured at near 200°C and melted at 228–229°C with decomposition. However, on rapid heating the melting point was raised up to 238—239°C. In alcoholic solution it was positive to the Mg-HCl reaction and also to the reaction with granulated zinc and hydrochloric acid. The mixed melting point with distylin showed the same behaviour with one of distylin itself.

Subst.	CO_2	H_2O	C %	H %
15.2 mg	33.4 mg	6.04 mg	59.95	3.95
Calc. for $C_{15}H_{12}O_7$			59.21	3.95

On the acetylation with acetic anhydride and pyridine the reduction product gave colourless needless, m.p. 153°C after three times of recrystallization from methanol. The methanol solution of the acetyl derivative was negative to ferric chloride solution, but positive to the Mg-HCl reaction. On the mixed melting point determination with pentaacetyl-distylin no depression was observed.

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